SEQ ID 1

ALIGNMENTS

```
RESULT   1
AAF57290
ID   AAF57290 standard; DNA; 3231 BP.
XX
AC   AAF57290;
XX
DT   29-MAY-2001  (first entry)
XX
DE   M. bovis Dalton 2d RTX toxin A subunit encoding DNA.
XX
KW   Moraxella; antigen; immune response; infection; RTX toxin; vaccine;
KW   antibacterial; ds.
XX
OS   Moraxella bovis.
XX
FH   Key
FT   CDS             Location/Qualifiers
FT                   232..3015
FT                   /*tag=  a
FT   CDS             /product= "RTX toxin A subunit"
FT                   1..195
FT                   /*tag=  b
FT   CDS             /note= "partial coding region of RTX toxin C subunit"
FT                   3080..3250
FT                   /*tag=  c
XX                   /note= "partial coding region of RTX toxin B subunit"
PN   WO200116172-A1.
XX
PD   08-MAR-2001.
XX
PF   31-AUG-2000; 2000WO-AU01048.
XX
```

BEST AVAILABLE COPY

PR 31-AUG-1999; 99AU-0002571.
XX
PA (CSIR) COMMONWEALTH SCI & IND RES ORG.
PA (UYME) UNIV MELBOURNE.
XX
PI Farn J, Strugnell R, Tennent J;
XX
DR WPI; 2001-235092/24.
DR P-PSDB; AAB62110, AAB62112, AAB62113.
XX
PT Novel Moraxella bovis antigen useful in compositions for raising immune
PT response in an animal, has protease, lipase or hemolysin activity -
XX
PS Claim 32; Fig 5; 60pp; English.
XX
CC The invention relates to new Moraxella bovis antigens and nucleic acid
CC sequences encoding these antigenic polypeptides. The antigenic
CC polypeptides and polynucleotides are useful for raising an immune
CC response in an animal directed against Moraxella, preferably against
CC M. bovis or M. catarrhalis, and for treating Moraxella infections. The
CC present sequence represents the nucleotide sequence of the A subunit of
CC the RTX toxin from M. bovis Dalton 2d.
XX
SQ Sequence 3231 BP; 1072 A; 478 C; 671 G; 1010 T; 0 other;

Query Match 99.9%; Score 2782.4; DB 22; Length 3231;
Best Local Similarity 100.0%; Pred. No. 0;
Matches 2783; Conservative 0; Mismatches 1; Indels 0; Gaps 0;

```
Qy      1 atgtccaatataaatgtaattaaatctaatattcaagcaggcttgaattcaacaaagtct 60
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    232 atgtccaatataaatgtaattaaatctaatattcaagcaggcttgaattcaacaaagtct 291

Qy     61 ggattaaaaaatctttacttggctattcccaaagattatgatccgcaaaaaggtgggact 120
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    292 ggattaaaaaatctttacttggctattcccaaagattatgatccgcaaaaaggtgggact 351

Qy    121 ttaaatgattttattaaagctgctgatgaattaggtattgctcgtttagcagaagagcct 180
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    352 ttaaatgattttattaaagctgctgatgaattaggtattgctcgtttagcagaagagcct 411

Qy    181 aatcacactgaaacagcaaaaaaatctgttgacacagtaaatcagtttctctctctcaca 240
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    412 aatcacactgaaacagcaaaaaaatctgttgacacagtaaatcagtttctctctctcaca 471

Qy    241 caaactggtattgctatttctgcaacaaaattagaaaagttcttacaaaaacattctacc 300
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    472 caaactggtattgctatttctgcaacaaaattagaaaagttcttacaaaaacattctacc 531

Qy    301 aataagttagccaaagggttagacagtgtagaaaatattgatcgtaaattaggtaaagca 360
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    532 aataagttagccaaagggttagacagtgtagaaaatattgatcgtaaattaggtaaagca 591

Qy    361 agtaatgtattatcaacattaagctctttttgggcactgcattagcgggtatagaacttt 420
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    592 agtaatgtattatcaacattaagctctttttgggcactgcattagcgggtatagaacttt 651

Qy    421 gattctttaatcaaaaaaggtgatgctgcacctgatgctttggctaaagctagtattgac 480
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    652 gattctttaatcaaaaaaggtgatgctgcacctgatgctttggctaaagctagtattgac 711

Qy    481 ttgattaatgagataattggtaatctatctcagagtactcaaacgattgaagcattttct 540
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    712 ttgattaatgagataattggtaatctatctcagagtactcaaacgattgaagcattttct 771

Qy    541 tcacagttagcaaagttaggttctactatatcgcaggctaaaggcttctctaatatagga 600
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    772 tcacagttagcaaagttaggttctactatatcgcaggctaaaggcttctctaatatagga 831

      601 aacaagttgcaaaacttaaatttttctaaaacaaatcttggtttggaaataattactggt 660
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      832 aacaagttgcaaaacttaaatttttctaaaacaaatcttggtttggaaataattactggt 891

Qy    661 ttgctatcaggcatttctgcaggctttgctttagcggataaaaatgcatcgactggcaaa 720
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    892 ttgctatcaggcatttctgcaggctttgctttagcggataaaaatgcatcgactggcaaa 951

Qy    721 aaagttgctgcaggttttgaattaagcaatcaagttattggtaatgtaacaaaagcaatt 780
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    952 aaagttgctgcaggttttgaattaagcaatcaagttattggtaatgtaacaaaagcaatt 1011

Qy    781 tcttcatatgttttagcacaacgtgttgctgctggtctatcaactactggtgctgttgct 840
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   1012 tcttcatatgttttagcacaacgtgttgctgctggtctatcaactactggtgctgttgct 1071

Qy    841 gctttaattacttcatcgattatgttggcaattagtccttttggcatttatgaatgcagca 900
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   1072 gctttaattacttcatcgattatgttggcaattagtccttttggcatttatgaatgcagca 1131

Qy    901 gataaattcaatcatgctaatgctcttgatgagtttgcaaaacaattccgaaaatttggc 960
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   1132 gataaattcaatcatgctaatgctcttgatgagtttgcaaaacaattccgaaaatttggc 1191

Qy    961 tatgatggggatcatttattggctgaatatcagcgtggtgtgggtactattgaagcttca 1020
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   1192 tatgatggggatcatttattggctgaatatcagcgtggtgtgggtactattgaagcttca 1251

Qy   1021 ttaactacaattagtacggcattaggtgcagtttctgctggtgtttccgctgctgctgta 1080
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   1252 ttaactacaattagtacggcattaggtgcagtttctgctggtgtttccgctgctgctgta 1311

Qy   1081 ggatctgctgttggtgcaccgattgcactattagttgcaggtgttacaggattgatctct 1140
          |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Db   1312 ggatctgctgttggtacaccgattgcactattagttgcaggtgttacaggattgatctct 1371

Qy   1141 ggaattttagaagcgtctaaacaggcaatgtttgaaagtgttgctaaccgtttacaaggt 1200
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   1372 ggaattttagaagcgtctaaacaggcaatgtttgaaagtgttgctaaccgtttacaaggt 1431

Qy   1201 aaaattttagagtgggaaaagcaaaatggcggtcagaactattttgataaaggctatgat 1260
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   1432 aaaattttagagtgggaaaagcaaaatggcggtcagaactattttgataaaggctatgat 1491

Qy   1261 tctcgttatgctgcttatttagctaataacttaaaatttttgtctgagctaaataaagag 1320
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   1492 tctcgttatgctgcttatttagctaataacttaaaatttttgtctgagctaaataaagag 1551

Qy   1321 ttggaagctgaacgtgttattgcaatcacccaacaacgttgggataataatattggtgag 1380
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   1552 ttggaagctgaacgtgttattgcaatcacccaacaacgttgggataataatattggtgag 1611

Qy   1381 ttagcaggtattaccaaattgggtgaacgcattaagagcggaaaagcttatgcagatgct 1440
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   1612 ttagcaggtattaccaaattgggtgaacgcattaagagcggaaaagcttatgcagatgct 1671

Qy   1441 tttgaagatggcaagaaagttgaagctggttccaatattactttggatgctaaaactggt 1500
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   1672 tttgaagatggcaagaaagttgaagctggttccaatattactttggatgctaaaactggt 1731

Qy   1501 atcatagacattagtaattcaaatgggaaaaaaacgcaagcgttgcatttcacttcgcct 1560
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   1732 atcatagacattagtaattcaaatgggaaaaaaacgcaagcgttgcatttcacttcgcct 1791

Qy   1561 ttgttaacagcaggaactgaatcacgtgaacgtttaactaatggtaaatactcttatatt 1620
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   1792 ttgttaacagcaggaactgaatcacgtgaacgtttaactaatggtaaatactcttatatt 1851

Qy   1621 aataagttaaaattcggacgtgtaaaaaactggcaagttacagatggagaggctagttct 1680
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   1852 aataagttaaaattcggacgtgtaaaaaactggcaagttacagatggagaggctagttct 1911

Qy   1681 aaattagatttctctaaagttattcagcgtgtagccgagacagaaggcacagacgagatt 1740
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   1912 aaattagatttctctaaagttattcagcgtgtagccgagacagaaggcacagacgagatt 1971
```

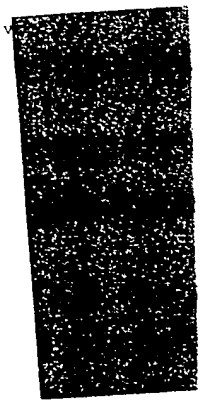
on Sep 16 16:43:25 2002

```
Qy   1741 ggtctaatagtaaatgcaaaagctggcaatgacgatatctttgttggtcaaggtaaaatg 1800
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   1972 ggtctaatagtaaatgcaaaagctggcaatgacgatatctttgttggtcaaggtaaaatg 2031

Qy   1801 aatattgatggtggagatggacacgatcgtgtcttctatagtaaagacggaggatttggt 1860
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   2032 aatattgatggtggagatggacacgatcgtgtcttctatagtaaagacggaggatttggt 2091

Qy   1861 aatattactgtagatggtacgagtgcaacagaagcaggcagttatacagttaatcgtaag 1920
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   2092 aatattactgtagatggtacgagtgcaacagaagcaggcagttatacagttaatcgtaag 2151

Qy   1921 gttgctcgaggtgatatctaccatgaagttgtgaagcgtcaagaaaccaaggtgggtaaa 1980
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   2152 gttgctcgaggtgatatctaccatgaagttgtgaagcgtcaagaaaccaaggtgggtaaa 2211

Qy   1981 cgtactgaaactatccagtatcgtgattatgaattaagaaaagttgggtatggttatcag 2040
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   2212 cgtactgaaactatccagtatcgtgattatgaattaagaaaagttgggtatggttatcag 2271

Qy   2041 tctaccgataatttgaaatcagtagaagaagtaattggttctcaatttaatgatgtattc 2100
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   2272 tctaccgataatttgaaatcagtagaagaagtaattggttctcaatttaatgatgtattc 2331

Qy   2101 aaaggttctaaattcaacgacatattccatagtggtgaaggtgatgatttactcgatggt 2160
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   2332 aaaggttctaaattcaacgacatattccatagtggtgaaggtgatgatttactcgatggt 2391

Qy   2161 ggtgctggtgacgaccgcttgtttggtggtaaaggcaacgatcgactttctggagatgaa 2220
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   2392 ggtgctggtgacgaccgcttgtttggtggtaaaggcaacgatcgactttctggagatgaa 2451

Qy   2221 ggcgatgatttactcgatggcggttctggtgatgatgtattaaatggtggtgctggtaat 2280
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   2452 ggcgatgatttactcgatggcggttctggtgatgatgtattaaatggtggtgctggtaat 2511

Qy   2281 gatgtctatatctttcggaaaggtgatggtaatgatactttgtacgatggcacgggcaat 2340
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   2512 gatgtctatatctttcggaaaggtgatggtaatgatactttgtacgatggcacgggcaat 2571

Qy   2341 gataaattagcatttgcagatgcaaatatatctgatattatgattgaacgtaccaaagag 2400
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   2572 gataaattagcatttgcagatgcaaatatatctgatattatgattgaacgtaccaaagag 2631

Qy   2401 ggtattatagttaaacgaaatgatcattcaggtagtattaacataccaagatggtacata 2460
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   2632 ggtattatagttaaacgaaatgatcattcaggtagtattaacataccaagatggtacata 2691

Qy   2461 acatcaaatttacaaaattatcaaagtaataaaacagatcataaaattgagcaactaatt 2520
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   2692 acatcaaatttacaaaattatcaaagtaataaaacagatcataaaattgagcaactaatt 2751

Qy   2521 ggtaaagatggtagttatatcacttccgatcaaattgataaaattttgcaagataagaaa 2580
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   2752 ggtaaagatggtagttatatcacttccgatcaaattgataaaattttgcaagataagaaa 2811

Qy   2581 gatggtacagtaattacatctcaagaattgaaaaagcttgctgatgagaataagagccaa 2640
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   2812 gatggtacagtaattacatctcaagaattgaaaaagcttgctgatgagaataagagccaa 2871

Qy   2641 aaattatctgcttcggacattgcaagtagcttaaataagctagttgggtcaatggcacta 2700
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   2872 aaattatctgcttcggacattgcaagtagcttaaataagctagttgggtcaatggcacta 2931

Qy   2701 tttggtacagcaaatagtgtgagttctaacgccttacagccaattacacaaccaactcaa 2760
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db   2932 tttggtacagcaaatagtgtgagttctaacgccttacagccaattacacaaccaactcaa 2991

Qy   2761 ggaattttggctccaagtgtttag 2784
          |||||||||||||||||||||||
Db   2992 ggaattttggctccaagtgtttag 3015
```

SEQ ID 6

ALIGNMENTS

RESULT 1
AAB62110
ID AAB62110 standard; Protein; 927 AA.
XX
AC AAB62110;
XX
DT 29-MAY-2001 (first entry)
XX
DE M. bovis Dalton 2d RTX toxin A subunit.
XX
KW Moraxella; antigen; immune response; infection; RTX toxin; v
KW antibacterial; A subunit.
XX
OS Moraxella bovis.
XX
PN WO200116172-A1.
XX
PD 08-MAR-2001.
XX
PF 31-AUG-2000; 2000WO-AU01048.
XX
PR 31-AUG-1999; 99AU-0002571.
XX
PA (CSIR) COMMONWEALTH SCI & IND RES ORG.
PA (UYME) UNIV MELBOURNE.
XX
PI Farn J, Strugnell R, Tennent J;
XX
DR WPI; 2001-235092/24.
DR N-PSDB; AAF57290.
XX
PT Novel Moraxella bovis antigen useful in compositions for raising immune
PT response in an animal, has protease, lipase or hemolysin activity
XX



88

PS Claim 26; Fig 5; 60pp; English.
XX
CC The invention relates to new Moraxella bovis antigens and nucleic acid
CC sequences encoding these antigenic polypeptides. The antigenic
CC polypeptides and polynucleotides are useful for raising an immune
CC response in an animal directed against Moraxella, preferably against
CC M. bovis or M. catarrhalis, and for treating Moraxella infections. The
CC present sequence represents the amino acid sequence of the A subunit of
CC the RTX toxin from M. bovis Dalton 2d.
XX
SQ Sequence 927 AA;

Query Match 100.0%; Score 57; DB 22; Length 927;
Best Local Similarity 100.0%; Pred. No. 0.2;
Matches 12; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 FLSELNKELEAE 12
||||||||||||
Db 433 flselnkeleae 444

SEQ 13

```
RESULT   1
AAB62110
ID   AAB62110 standard; Protein; 927 AA.
XX
AC   AAB62110;
XX
DT   29-MAY-2001  (first entry)
XX
DE   M. bovis Dalton 2d RTX toxin A subunit.
XX
KW   Moraxella; antigen; immune response; infection; RTX toxin; vaccine;
KW   antibacterial; A subunit.
XX
OS   Moraxella bovis.
XX
PN   WO200116172-A1.
XX
PD   08-MAR-2001.
XX
PF   31-AUG-2000; 2000WO-AU01048.
XX
PR   31-AUG-1999;   99AU-0002571.
XX
PA   (CSIR ) COMMONWEALTH SCI & IND RES ORG.
PA   (UYME ) UNIV MELBOURNE.
XX
PI   Farn J,  Strugnell R,  Tennent J;
XX
DR   WPI; 2001-235092/24.
DR   N-PSDB; AAF57290.
XX
PT   Novel Moraxella bovis antigen useful in compositions for raising immune
PT   response in an animal, has protease, lipase or hemolysin activity  -
XX
PS   Claim 26; Fig 5; 60pp; English.
XX
CC   The invention relates to new Moraxella bovis antigens and nucleic acid
CC   sequences encoding these antigenic polypeptides. The antigenic
CC   polypeptides and polynucleotides are useful for raising an immune
CC   response in an animal directed against Moraxella, preferably against
CC   M. bovis or M. catarrhalis, and for treating Moraxella infections. The
CC   present sequence represents the amino acid sequence of the A subunit of
CC   the RTX toxin from M. bovis Dalton 2d.
XX
SQ   Sequence   927 AA;


  Query Match             100.0%;  Score 77;  DB 22;  Length 927;
  Best Local Similarity   100.0%;  Pred. No. 7.4e-05;
  Matches   14;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy       1 FNDIFHSGEGDDLL 14
           ||||||||||||||
Db     705 fndifhsgegddll 718
```

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 March 2001 (08.03.2001)

PCT

(10) International Publication Number
WO 01/16172 A1

(51) International Patent Classification[7]: C07K 14/22, 16/12, A61K 38/48, 39/095, C12N 15/31

(21) International Application Number: PCT/AU00/01048

(22) International Filing Date: 31 August 2000 (31.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PQ 2571 31 August 1999 (31.08.1999) AU

(71) Applicants *(for all designated States except US)*: **COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION** [AU/AU]; Limestone Avenue, Campbell, ACT 2601 (AU). **THE UNIVERSITY OF MELBOURNE** [AU/AU]; Royal Parade, Parkville, VIC 3052 (AU).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **FARN, Jacinta** [AU/AU]; 61 Glass Street, Essendon, VIC 3040 (AU). **STRUGNELL, Richard** [AU/AU]; 17 Salisbury Grove, Hawthorn, VIC 3122 (AU). **TENNENT, Jan** [AU/AU]; 6 Campbell Grove, Hawthorn East, VIC 3123 (AU).

(74) Agent: **F B RICE & CO**; 139 Rathdowne Street, Carlton, VIC 3053 (AU).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/16172 A1

(54) Title: VACCINE ANTIGENS OF MORAXELLA

(57) Abstract: The present invention relates to antigens of *Moraxella*, in particular, *Moraxella bovis*, nucleic acid sequences encoding these antigens and formulations for use in raising an immune response against *Moraxella*.

Vaccine antigens of Moraxella

FIELD OF THE INVENTION

The present invention relates to antigens of *Moraxella*, in particular, *Moraxella bovis*, nucleic acid sequences encoding these antigens and formulations for use in raising an immune response against *Moraxella*.

BACKGROUND OF THE INVENTION

Infectious bovine keratoconjunctivitis (IBK) is an economically important disease of cattle caused by the Gram-negative coccobacillus *Moraxella bovis*. More commonly known as pinkeye, IBK is a highly contagious ocular infection which may range from mild conjunctivitis to severe ulceration, corneal perforation and blindness. Therapeutic and preventative measures have limited success in controlling IBK and a vaccine which will prevent the disease is required. A number of factors contribute to the virulence of the organism, the two most important attributes so far identified are the expression of pili, and the ability to produce haemolysin. Seven different serogroups of *M. bovis* strains isolated in Australia, Great Britain and the USA have been characterised, based on pilus types (1). An efficacious pilus-based vaccine must contain a sufficient quantity of pili from all seven serotypes to be fully protective, because of a lack of cross protection between serotypes (2, 3). Expression of all seven pilus serotypes at levels high enough to be useful for commercial vaccine preparation has not been achieved.

The ideal vaccine candidate to stimulate protection against *M. bovis* would be a molecule that is highly-conserved among all strains of this species. Possible candidates are haemolysin, protease, lipase and/or phospholipase (4) enzymes produced by *M. bovis*. For example, a partially purified cell-free supernatant from one haemolytic strain of *M. bovis* has been shown to confer significant protection against heterologous, wild-type challenge (5). The possibility that a haemolysin could be conserved across all seven serotypes of *M. bovis* makes it a potential vaccine candidate against IBK. However, researchers have so far been unable to either clone the gene encoding the haemolysin or purify the protein to homogeneity. Nevertheless, any or all of these molecules, alone or in combination, could prove useful for the generation of an effective vaccine against IBK.

SUMMARY OF THE INVENTION

In a first aspect the present invention consists in a polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO. 1 from amino acid 37 to 1114, or a sequence having at least 50% identity thereto, or a functional fragment thereof.

In a preferred embodiment the polypeptide has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 1.

In a further preferred embodiment of the first aspect of the present invention the polypeptide has protease activity.

In a second aspect the present invention consists in a nucleic acid molecule, the nucleic acid molecule encoding the polypeptide of the first aspect of the present invention.

In a third aspect the present invention consists in a nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO. 2 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

In a preferred embodiment the nucleic acid molecule has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 2.

In a fourth aspect the present invention consists in a composition for use in raising an immune response in an animal, the composition comprising the polypeptide of the first aspect of the present invention or the nucleic acid sequence of the second aspect of the present invention and optionally a carrier and/or adjuvant.

In a fifth aspect the present invention consists in a polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO. 3 from amino acid 26 to 616, or a sequence having at least 50% identity thereto, or a functional fragment thereof.

In a preferred embodiment the polypeptide has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 3 from amino acid 26 to 616.

In a further preferred embodiment of the fifth aspect the polypeptide has lipase activity.

In a sixth aspect the present invention consists in a nucleic acid molecule, the nucleic acid molecule encoding the polypeptide of the fifth aspect of the present invention.

In a seventh aspect the present invention consists in a nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO. 4 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

In a preferred embodiment the nucleic acid molecule has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 4.

In an eighth aspect the present invention consists in a composition for use in raising an immune response in an animal, the composition comprising the polypeptide of the fifth aspect of the present invention or the nucleic acid sequence of the sixth aspect of the present invention and optionally a carrier and/or adjuvant.

In a ninth aspect the present invention consists in a polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO. 5, or a sequence having at least 60% identity thereto, or a functional fragment thereof.

In a preferred embodiment the polypeptide has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 5.

In a further preferred embodiment of the ninth aspect the polypeptide has haemolysin activity.

In a tenth aspect the present invention consists in a nucleic acid molecule, the nucleic acid molecule encoding the polypeptide of the ninth aspect of the present invention.

In an eleventh aspect the present invention consists in a nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO. 6 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

In a preferred embodiment the nucleic acid molecule has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 6.

In a twelfth aspect the present invention consists in a composition for use in raising an immune response in an animal, the composition comprising

the polypeptide of the ninth aspect of the present invention or the nucleic acid sequence of the tenth aspect of the present invention and optionally a carrier and/or adjuvant.

The term "functional fragment" as used herein is intended to cover fragments of the polypeptide which retain at least 10% of the biological activity of the complete polypeptide. In particular this term is used to encompass fragments which show immunological cross-reactivity with the entire polypeptide, eg ligands which react with the fragment also react with the complete polypeptide.

In a thirteenth aspect the present invention consists in a composition for use in raising an immune response in an animal directed against *Moraxella*, the composition comprising at least one polypeptide selected from the group consisting of the polypeptides of the first, fifth and ninth aspects of the present invention and optionally including an adjuvant or carrier.

In a preferred embodiment the composition includes the polypeptide of the ninth aspect of the present invention and either one of, or preferably both, the polypeptides of the first and fifth aspects of the present invention.

In a preferred embodiment the *Moraxella* is *M. bovis or M. catarrhalis*, most preferably *M. bovis*.

In a fourteenth aspect the present invention consists in an antibody raised against a polypeptide selected from the group consisting of the polypeptides of the first, fifth and ninth aspects.

As will be readily appreciated by the person skilled in this field the polypeptides and antibodies of the present invention and probes derived from the nucleotide sequences can be used as diagnostic reagents in determining *Moraxella*, in particular, *M. bovis* infection. For example, the polypeptides and antibodies can be used in ELISA based assays whilst the probes can be used in PCR based assays. The probes will be of a length to provide the required level of specificity and will typically be at least 18 nucleotides in length.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Nucleotide and amino acid sequence of a protease from *M. bovis* Dalton 2d. A putative promoter sequence is singly underlined. A putative ribosome binding site is shown in bold and underlined. A putative start codon is shown in bold. Putative transcription terminator sequences are indicated by inverted arrows. Numbering for both the nucleotide and amino acid sequences are shown on the left hand side.

Figure 2: Nucleotide and amino acid sequence of a lipase from *M. bovis* Dalton 2d. A putative promoter sequence is singly underlined. A putative ribosome binding site is shown in bold and underlined. A putative start codon is shown in bold. Putative transcription terminator sequences are indicated by inverted arrows. Numbering for both the nucleotide and amino acid sequences are shown on the left hand side.

Figure 3: Heat stability of the lipase from *M.bovis* when expressed in its recombinant form (pMB1/MC1061). (Heating carried out at 90°C).

Figure 4: Comparison of growth rate and expression levels of the lipase of *M.bovis* when in its (i) native form and (ii) recombinant form. The growth rate is shown as solid bars and the lipase expression levels as open diamonds.

Figure 5: Nucleotide and amino acid sequence of the A subunit of the RTX toxin from *M. bovis* Dalton 2d. A putative ribosome binding site is shown in bold and underlined. A putative start codon is shown in bold. Upstream of the A subunit open reading frame is a portion of the coding region for the C subunit (nucleotide 1 to 195) (corresponding amino acid sequence shown in SEQ ID NO:8) and downstream of the A subunit is a small portion of the B subunit coding region (nucleotide 3080 to 3250) (corresponding amino acid sequence shown in SEQ ID NO:9) . Numbering for both the nucleotide and amino acid sequences are shown on the left hand side.

DETAILED DESCRIPTION OF THE INVENTION

In order that the nature of the present invention may be more clearly understood preferred forms thereof will now be described with reference to the following non-limiting Examples.

General Molecular Biology

Unless otherwise indicated, the recombinant DNA techniques utilized in the present invention are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel et al. (Editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present) and are incorporated herein by reference.

Protein Variants

Amino acid sequence variants can be prepared by introducing appropriate nucleotide changes into DNA, or by *in vitro* synthesis of the desired polypeptide. Such variants include, for example, deletions, insertions or substitutions of residues within the amino acid sequence. A combination of deletion, insertion and substitution can be made to arrive at the final construct, provided that the final protein product possesses the desired characteristics. The amino acid changes also may alter post-translational processes such as altering the membrane anchoring characteristics, altering the intra-cellular location by inserting, deleting or otherwise affecting the transmembrane sequences of the native protein, or modifying its susceptibility to proteolytic cleavage.

In designing amino acid sequence variants, the location of the mutation site and the nature of the mutation will depend on characteristic(s) to be modified. The sites for mutation can be modified individually or in

series, e.g., by (1) substituting first with conservative amino acid choices and then with more radical selections depending upon the results achieved, (2) deleting the target residue, or (3) inserting residues of other ligands adjacent to the located site.

A useful method for identification of residues or regions for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (*Science* (1989) **244**: 1081-1085*)*. Here, a residue or group of target residues are identified (e.g., charged residues such as Arg. Asp, His, Lys, and Glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the surrounding aqueous environment in or outside the cell. Those domains demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to optimise the performance of a mutation at a given site, alanine scanning or random mutagenesis may be conducted at the target codon or region and the expressed variants are screened for the optimal combination of desired activity.

There are two principal variables in the construction of amino acid sequence variants; the location of the mutation site and the nature of the mutation. These may represent naturally occurring alleles or predetermined mutant forms made by mutating the DNA either to arrive at an allele or a variant not found in nature. In general, the location and nature of the mutation chosen will depend upon the characteristic to be modified.

Amino acid sequence deletions generally range from about 1 to 30 residues, more preferably about 1 to 10 residues and typically about 1 to 5 contiguous residues.

Amino acid sequence insertions include amino and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Other insertional variants include the fusion of the N- or C-terminus of the proteins to an immunogenic polypeptide e.g. bacterial polypeptides such as betalactamase or an enzyme encoded by the *E. coli trp* locus, or yeast protein, bovine serum albumin, and chemotactic polypeptides. C-terminal fusions with proteins having a

long half-life such as immunoglobulin constant regions (or other immunoglobulin regions), albumin, or ferritin, are included.

Another group of variants are amino acid substitution variants. These variants have at least one amino acid residue in the protein molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include sites identified as the active site(s). Other sites of interest are those in which particular residues obtained from various species are identical. These positions may be important for biological activity. These sites, especially those falling within a sequence of at least three other identically conserved sites, are substituted in a relatively conservative manner. Such conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 1, or as further described below in reference to amino acid classes, are introduced and the products screened.

TABLE 1

Original Residue	**Exemplary Substitutions**	**Preferred Substitutions**
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; lys; arg	gln
Asp (D)	glu	glu
Cys (C)	ser	ser
Gln (Q)	asn	asn
Glu (E)	asp	asp
Gly (G)	pro	pro
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe norleucine	leu
Leu (L)	norleucine, ile; val; met; ala; phe	ile

Original Residue	Exemplary Substitutions	Preferred Substitutions
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile;	leu
Phe (F)	leu; val; ile; ala	leu
Pro (P)	gly	gly
Ser (S)	thr	thr
Thr (T	ser	ser
Trp (W)	tyr	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

Mutants, Variants and Homology - Proteins

Mutant polypeptides will possess one or more mutations which are deletions, insertions, or substitutions of amino acid residues. Mutants can be either naturally occurring (that is to say, purified or isolated from a natural source) or synthetic (for example, by performing site-directed mutagensis on the encoding DNA). It is thus apparent that polypeptides of the invention can be either naturally occurring or recombinant (that is to say prepared using recombinant DNA techniques).

An allelic variant will be a variant that is naturally occurring within an individual organism.

Protein sequences are homologous if they are related by divergence from a common ancestor. Consequently, a species homologue of the protein will be the equivalent protein which occurs naturally in another species. Within any one species a homologue may exist as numerous allelic variants, and these will be considered homologues of the protein. Allelic variants and species homologues can be obtained by following standard techniques known to those skilled in the art. Preferred species homologues include those obtained from representatives of the same Phylum, more preferably the same Class and even more preferably the same Order.

A protein at least 50% identical, as determined by methods well known to those skilled in the art (for example, the method described by Smith, T.F. and Waterman, M.S. (1981) Ad. Appl Math., 2: 482-489, or Needleman, S.B. and Wunsch, C.D. (1970) J. Mol. Biol., 48: 443-453), to that of the present invention are included in the invention, as are proteins at least 70% or 80% and more preferably at least 90% identical to the protein of the present invention. This will generally be over a region of at least 20, preferably at least 30, contiguous amino acids.

Mutants, Variants and Homology - Nucleic Acids

Mutant polynucleotides will possess one or more mutations which are deletions, insertions, or substitutions of nucleotide residues. Mutants can be either naturally occurring (that is to say, isolated from a natural source) or synthetic (for example, by performing site-directed mutagensis on the DNA). It is thus apparent that polynucleotides of the invention can be either naturally occurring or recombinant (that is to say prepared using recombinant DNA techniques).

An allelic variant will be a variant that is naturally occurring within an individual organism.

Nucleotide sequences are homologous if they are related by divergence from a common ancestor. Consequently, a species homologue of the polynucleotide will be the equivalent polynucleotide which occurs naturally in another species. Within any one species a homologue may exist as numerous allelic variants, and these will be considered homologues of the polynucleotide. Allelic variants and species homologues can be obtained by following standard techniques known to those skilled in the art. Preferred species homologues include those obtained from representatives of the same Phylum, more preferably the same Class and even more preferably the same Order.

A polynucleotide at least 70% identical, as determined by methods well known to those skilled in the art (for example, the method described by Smith, T.F. and Waterman, M.S. (1981) Ad. Appl Math., 2: 482-489, or Needleman, S.B. and Wunsch, C.D. (1970) J. Mol. Biol., 48: 443-453), to that of the present invention are included in the invention, as are proteins at least 80% or 90% and more preferably at least 95% identical to the

polynucleotide of the present invention. This will generally be over a region of at least 60, preferably at least 90, contiguous nucleotide residues.

Antibody Production

The term "antibody" should be construed as covering any specific binding substance having a binding domain with the required specificity. Thus, the term covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including any polypeptide including an immunoglobulin binding domain, whether natural or synthetic. Chimaeric molecules including an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included.

Antibodies, either polyclonal or monoclonal, which are specific for a protein of the present invention can be produced by a person skilled in the art using standard techniques such as, but not limited to, those described by Harlow et al. Antibodies: A Laboratory Manual, Cold Springs Harbor Laboratory Press (1988), and D. Catty (editor), Antibodies: A Practical Approach, IRL Press (1988).

Various procedures known in the art may be used for the production of polyclonal antibodies to epitopes of a protein. For the production of polyclonal antibodies, a number of host animals are acceptable for the generation of antibodies by immunization with one or more injections of a polypeptide preparation, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response in the host animal, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminium hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, oil emulsions, keyhole lympet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

A monoclonal antibody to an epitope of a protein may be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein (1975, Nature 256, 493-497), and the more recent human B-cell hybridoma technique (Kesber *et al.* 1983, Immunology Today 4:72) and EBV-hybridoma technique (Cole *et al.* 1985, Monoclonal Antibodies and

Cancer Therapy, Alan R. Liss, Inc. pp. 77-96). In addition, techniques developed for the production of "chimeric antibodies" by splicing the genes from an antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity may be used (Morrison *et al.* 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger *et al.* 1984 Nature 312:604-608; Takeda *et al.* 1985 Nature 31:452-454). Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce 4-specific single chain antibodies.

Recombinant human or humanized versions of monoclonal antibodies are a preferred embodiment for human therapeutic applications. Humanized antibodies may be prepared according to procedures in the literature (e.g. Jones *et al.* 1986, Nature 321:522-25; Reichman *et al.* 1988, Nature 332:323-27; Verhoeyen *et al.* 1988, Science 239:1534-36). The recently described "gene conversion mutagenesis" strategy for the production of humanized monoclonal antibody may also be employed in the production of humanized antibodies (Carter *et al.* 1992 Proc. Natl. Acad. Sci. U.S.A. 89:4285-89). Alternatively, techniques for generating the recombinant phage library of random combinations of heavy and light regions may be used to prepare recombinant antibodies (e.g. Huse *et al.* 1989 Science 246:1275-81).

Antibody fragments which contain the idiotype of the molecule such as Fu $F(ab^1)$ and $F(ab^2)$ may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab) E2 fragment which can be produced by pepsin digestion of the intact antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab')2 fragment, and the two Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent. Alternatively, Fab expression libraries may be constructed (Huse *et al.* 1989, Science 246:1275-1281) to allow rapid and easy cloning of a monoclonal Fab fragment with the desired specificity to a protein.

Adjuvants and Carriers

Pharmaceutically acceptable carriers or diluents include those used in compositions suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. They are

non-toxic to recipients at the dosages and concentrations employed. Representative examples of pharmaceutically acceptable carriers or diluents include, but are not limited to water, isotonic solutions which are preferably buffered at a physiological pH (such as phosphate-buffered saline or Tris-buffered saline) and can also contain one or more of, mannitol, lactose, trehalose, dextrose, glycerol, ethanol or polypeptides (such as human serum albumin). The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy.

As mentioned above the composition may include an adjuvant. As will be understood an "adjuvant" means a composition comprised of one or more substances that enhances the immunogenicity and efficacy of a vaccine composition. Non-limiting examples of suitable adjuvants include squalane and squalene (or other oils of animal origin); block copolymers; detergents such as Tween®-80; Quil® A, mineral oils such as Drakeol or Marcol, vegetable oils such as peanut oil; *Corynebacterium*-derived adjuvants such as *Corynebacterium parvum*; *Propionibacterium*-derived adjuvants such as *Propionibacterium acne*; *Mycobacterium bovis* (Bacille Calmette and Guerin or BCG); interleukins such as interleukin 2 and interleukin 12; monokines such as interleukin 1; tumour necrosis factor; interferons such as gamma interferon; combinations such as saponin-aluminium hydroxide or Quil-A aluminium hydroxide; liposomes; ISCOM adjuvant; mycobacterial cell wall extract; synthetic glycopeptides such as murarmyl dipeptides or other derivatives; Avridine; Lipid A derivatives; dextran sulfate; DEAE-Dextran or with aluminium phosphate; carboxypolymethylene such as Carbopol' EMA; acrylic copolymer emulsions such as Neocryl A640 (e.g. U.S. Pat. No. 5,047,238); vaccinia or animal poxvirus proteins; sub-viral particle adjuvants such as cholera toxin, or mixtures thereof.

Gene/DNA Isolation

The DNA encoding a protein may be obtained from any cDNA library prepared from tissue believed to express the gene mRNA and to express it at a detectable level. DNA can also be obtained from a genomic library.

Libraries are screened with probes or analytical tools designed to identify the gene of interest or the protein encoded by it. For cDNA expression libraries, suitable probes include monoclonal or polyclonal

antibodies that recognize and specifically bind the protein; oligonucleotides of about 20-80 bases in length that encode known or suspected portions of cDNA from the same or different species; and/or complementary or homologous cDNAs or fragments thereof that encode the same or a hybridizing gene. Appropriate probes for screening genomic DNA libraries include, but are not limited to, oligonucleotides; cDNAs or fragments thereof that encode the same or hybridizing DNA including expressed sequence tags and the like; and/or homologous genomic DNAs or fragments thereof. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*

An alternative means to isolate a gene encoding the protein of interest is to use polymerase chain reaction (PCR) methodology as described in section 14 of Sambrook *et al.* This method requires the use of oligonucleotide probes that will hybridize to the gene.

The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The actual nucleotide sequence(s) is usually based on conserved or highly homologous nucleotide sequences or regions of the gene. The oligonucleotides may be degenerate at one or more positions. The use of degenerate oligonucleotides may be of particular importance where a library is screened from a species in which preferential codon usage in that species is known. The oligonucleotide must be labelled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of labelling is to use (α-^{32}P)- dATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labelling.

DNA encompassing all the protein coding sequence is obtained by screening selected cDNA or genomic libraries, and if necessary, using conventional primer extension procedures as described in section 7.79 of Sambrook *et al.*, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

Another alternative method for obtaining the gene of interest is to chemically synthesize it using one of the methods described in Fingels *et al.* (*Agnew Chem. Int. Ed. Engl. 28:* 716-734, 1989). These methods include

triester, phosphite, phosphoramidite and H-Phosphonate methods, PCR and other autoprimer methods, and oligonucleotide syntheses on solid supports. These methods may be used if the entire nucleic acid sequence of the gene is known, or the sequence of the nucleic acid complementary to the coding strand is available, or alternatively, if the target amino acid sequence is known, one may infer potential nucleic acid sequences using known and preferred coding residues for each amino acid residue.

Substantially Purified

By "substantially purified" we mean a polypeptide that has been separated from lipids, nucleic acids, other polypeptides, and other contaminating molecules.

Hybridisation

The polynucleotide sequence of the present invention may hybridise to the respective sequence set out SEQ. ID. NOS. 2, 4, or 6 under high stringency. As used herein, stringent conditions are those that (i) employ low ionic strength and high temperature for washing after hybridization, for example, 0.1 x SSC and 0.1% (w/v) SDS at 50°C; (ii) employ during hybridization conditions such that the hybridization temperature is 25°C lower than the duplex melting temperature of the hybridizing polynucleotides, for example 1.5 x SSPE, 10% (w/v) polyethylene glycol 6000, 7% (w/v) SDS, 0.25 mg/ml fragmented herring sperm DNA at 65°C; or (iii) for example, 0.5M sodium phosphate, pH 7.2, 5mM EDTA, 7% (w/v) SDS and 0.5% (w/v) BLOTTO at 70°C; or (iv) employ during hybridization a denaturing agent such as formamide, for example, 50% (v/v) formamide with 5 x SSC, 50mM sodium phosphate (pH 6.5) and 5 x Denhardt's solution (32) at 42°C; or (v) employ, for example, 50% (v/v) formamide, 5 x SSC, 50mM sodium phosphate (pH 6.8), 0.1% (w/v) sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50μg/ml) and 10% dextran sulphate at 42°C.

EXAMPLE 1

This example describes the cloning and characterisation of a protease from *Moraxella bovis*.

Bacteria and construction of a genomic library

Moraxella bovis strain Dalton 2d was a field isolate collected from a bovine eye and characterised by CSIRO Animal Health, Parkville, Australia (6). *Escherichia coli* strain DH5α has been previously described (7, 8).

All enzymes were purchased from Promega (Madison, WI, USA) except where otherwise noted.

General cloning and DNA techniques were as described (9) unless otherwise noted.

A genomic library was constructed by carrying out partial *Sau*3A digests on genomic DNA extracted from *M. bovis* strain Dalton 2d using a CTAB method which is outlined below. This DNA was size fractionated using a NaCl gradient (10) and ligated with the cosmid cloning vector pHC79 (11) which had been previously digested with *Bam*HI. This DNA was packaged into lambda bacteriophage heads using the Packagene Lambda DNA packaging system (Promega, Madison,WI, USA) and this was used to transduce the *E. coli* strain DH5α. The library was stored in 96 well trays (50% glycerol / luria broth / ampicillin (50μg/ml)) at -70°C.

CTAB genomic DNA extraction from *M. bovis*

A 5ml brain heart infusion (BHI) (Oxoid Ltd., Basingstoke, Hampshire, U.K.) broth was inoculated with a colony of Dalton 2d taken from a fresh overnight culture on horse blood agar and incubated with shaking at 37°C for 6 hours. This culture was used to inoculate 50ml of BHI broth which was grown with shaking at 37°C overnight. 40ml of the culture was transferred to an SS34 tube and the cells pelleted at 3000 × g for 10 minutes. Following resuspension of the pellet in 9.5ml of 25% sucrose in TE buffer (10mM Tris, 1mM EDTA (pH8)), 500μl of 10% SDS, 50μl of 20mg/ml proteinase K and 20μl of 10mg/ml RnaseA were added and this mixture incubated in an orbital shaker for 1 hour at 37°C. To this mixture, 1.8ml of 5M NaCl and 1.5ml of a CTAB (N-Cetyl-N,N,N-trimethyl-ammonium bromide) / NaCl solution was added and incubation continued for 20 minutes at 65°C. The DNA was

extracted using phenol/chloroform and precipitated with 0.6 volumes of isopropanol. The resulting DNA was washed in 70% ethanol, dried and resuspended in 2ml of TE buffer.

Screening of genomic library for enzyme activity

The genomic library was cultured on skim milk agar to screen for the presence of a clone displaying protease activity (double strength Columbia agar base (Oxoid Ltd., Basingstoke, Hampshire, U.K.) / 10% skim milk) for 24 hours at 37°C followed by refrigeration at 4°C for one to two days.

A single clone from the genomic library was detected as having activity against skim milk agar. DNA analysis confirmed that the clone contained a fragment of *M. bovis* Dalton 2d genomic DNA approximately 40 kilobases in size. The construct was designated pJF1.

Nucleotide sequence of the *M. bovis* protease clone pJF1

Plasmid and cosmid DNA for automated sequencing was extracted using the Wizard Plus SV Minipreps DNA Purification System (Promega, Madison, WI, USA) and the Qiagen Plasmid Midi Kit (Qiagen Pty. Ltd., Clifton Hill, Vic, Australia), respectively.

The nucleotide sequence of the insert DNA was determined using the process of "primer walking" (12). This was achieved using synthetic oligonucleotides (Bresatec / Geneworks, Thebarton, SA, Australia) and the dye terminator cycle sequencing ready reaction (Perkin Elmer Corporation, Norwalk, CT, USA). The resulting sequence was analysed on an Applied Biosystems 373A DNA sequencer.

Automated sequencing revealed an open reading frame of 3345bp capable of encoding a protein of 1115 amino acids. The sequence is written in the 5' to 3' direction and is shown in Figure 1 together with the corresponding amino acid sequence which is predicted to encode a protein with a molecular weight of 120kDa. The amino acid sequence is shown in SEQ. ID. NO. 1 and the DNA sequence is shown in SEQ. ID. NO. 2.

The putative start codon for the mature protease protein was identified by the presence of a possible ribosome binding site upstream. This RBS was identified by its similarity to the consensus sequence for the *E. coli* RBS and that previously identified for the *M. bovis* pilin genes (AGGAG) (27)

Due to the secreted nature of the protease, it was assumed that it would contain in its N-terminal sequence a signal peptide which would be used in the secretion of the protein. This analysis was carried out using a prediction program (SignalP) available through the Expasy website (http://www.expasy.ch/tools/), which allows for the identification of prokaryotic signal peptides and predicts possible cleavage sites. This analysis only identified a signal peptide using the start codon indicated in the accompanying protein/DNA sequence.

Sequence comparisons

Comparisons of the deduced amino acid sequence with those in the database were carried out using the BlastX and BlastP programs (13) which are available at http://www.ncbi.nlm.nih.gov.

At the amino acid level, the protease cloned from Dalton 2d displayed the following similarity and identity to the proteins listed.

Organism	Protein	Similarity	Identity
Serratia marcescens	ssp-h2 - serine protease autotransporter	39%	23%
Serratia marcescens	ssp-h1 - serine protease autotransporter	37%	22%
Pseudomonas flourescens	serine protease homologue	34%	20%
Pseudomonas tolaasii	serine protease	35%	21%

More generally the 5' domain of the *M. bovis* protease displays homology to a family of subtilisins (serine proteases) while the 3' region resembles a number of outer membrane proteins.

The *M. bovis* sequence was found to contain a highly proline rich region which distinguished it from all other proteins to which it was closely related.

Protease type encoded by pJF1

In order to identify the type of protease activity encoded by pJF1, a range of specific protease inhibitors were examined for their effect on the expression of the *M. bovis* protease.

The method of Bourgeau *et al.*, (1992) (14) was used to determine inhibitor activity with the following modifications. 100µl of cell free supernatant from a fresh overnight broth culture was mixed with 650µl of 100mM Tris (pH 7.2) and a suitable volume of inhibitor PMSF (phenylmethylsulfonyl fluoride) 5mM; EDTA 5mM; leupeptin 100µg/ml; pepstatin 50µg/ml]. Distilled water was used to make the volume up to 1ml. The mixture was incubated at 37°C for 30 minutes and 10mg of azocoll (Calbiochem, Alexandria, NSW, Australia) was then added. The suspensions were incubated at 37°C for 16 hours and the optical density read at 520nm.

In this way it was confirmed that the activity attributable to the protease encoded by pJF1 was that of a serine protease since PMSF (a serine protease inhibitor) reduced the protease activity of both Dalton 2d and pJF1 to zero.

Conservation of protease in *M. bovis*

Southern hybridisation using an internal fragment of the protease coding region as a probe was carried out to investigate whether the protease was present in strains representing the known *M. bovis* pili serotypes.

Genomic DNA extracted from the representative strains of *M. bovis* (15) was digested with *Xba*I and *Eco*RI and separated using agarose gel electrophoresis. The DNA was transferred to a Hybond N+ filter (Amersham, Little Chalfont, Buckinghamshire, U.K.) using the method described (9). The probe used in the southern hybridisation was a PCR amplified fragment which was internal to the protease coding region. This fragment was labelled with α^{32}P-dATP using the Megaprime labelling system (Amersham, Little Chalfont, Buckinghamshire, U.K.) according to the manufacturers instructions. High stringency conditions were used (hybridisation temperature 68°C; 2 washes at room temperature in 2 × SSC / 0.1% SDS; 1 wash at 68°C in 0.1 × SSC / 0.1% SDS) and the resulting filters were exposed to autoradiographic film (Kodak, Rochester, New York, USA) for 5 to 24 hours before developing.

Results showed that the protease gene cloned in pJF1 is present in all strains of *M. bovis* examined.

EXAMPLE 2

This example describes the cloning and characterisation of a lipase from *Moraxella bovis*.

Bacteria and construction of a plasmid library

Moraxella bovis strain Dalton 2d was a field isolate collected from a bovine eye and characterised by CSIRO Animal Health, Parkville, Australia (6). *Escherichia coli* strain MC1061 has been previously described (16).

All enzymes were purchased from Promega (Madison, WI, USA) except where otherwise noted. General cloning and DNA techniques were as described (9) unless otherwise noted.

A plasmid library was constructed in the cloning vector pBR322 (17). This was done by partially digesting genomic DNA extracted from Dalton 2d (using the CTAB method described in Example 1) with *Sau*3A under conditions that maximised the amount of DNA in the range of 1 to 2kb. This DNA was ligated with pBR322 which had been previously digested with *Bam*HI. The ligated DNA was electroporated (2.5kV, 200Ω and 200μF, for a theoretical time constant of 4.7) into electrocompetent *E. coli* MC1061 cells.

Screening of plasmid library for lipase expression

Following electroporation of the ligated DNA into MC1061 cells, recombinant clones displaying lipase activity were detected by culturing the library for 24 hours at 37°C on media containing Tween 80 [10ml Tween 80 (Sigma, St Louis, MO, USA), 5g NaCl, 3g agar No.1 (Oxoid Ltd., Basingstoke, Hampshire, U.K.), 10g peptone, 0.1g $CaCl_2.H_20$ / litre].

Twenty eight out of 24,000 clones screened were found to be displaying lipase activity. DNA analysis confirmed that all of these clones contained one 5.4kb fragment of DNA in common. One clone was chosen to continue work with and this was designated pMB1.

In some experiments (below), a photometric assay of extracellular lipase activity was performed with p-nitrophenylpalmitate as the substrate (18, 19). Strains of *E. coli* and/or *M. bovis* were grown at 37°C for the required time points. Cell free culture supernatant (100μl) was mixed with 2.4ml of enzyme buffer (19) to assay secreted lipase activity. After 30 minutes incubation at 37°C, the optical density at 410nm was determined.

One enzyme unit was defined as the amount of enzyme that releases 1 nmol of p-nitrophenyl from p-nitrophenylpalmitate ml^{-1} min^{-1}. Under the conditions described by Stuer *et al.*, (18), an optical density at 410nm of 0.041 is equivalent to 1 enzyme unit.

Nucleotide sequence of the *M. bovis* lipase clone pMB1

Plasmid pMB1 was subjected to automated DNA sequencing using the methodology described in Example 1.

This analysis revealed an open reading frame of 1851bp capable of encoding 617 amino acids. The sequence is written in the 5' to 3' direction and is shown in Figure 2 together with the corresponding amino acid sequence that is predicted to encode a protein with a molecular weight of 65.8kDa. The amino acid sequence is shown in SEQ. ID. NO. 3 and the DNA sequence is shown in SEQ. ID. NO. 4.

The techniques set out above in respect of the protease were used to identify the potential start codon for the lipase protein.

Sequence comparisons

Sequence comparisons were made using the methodology described in Example 1.

At the amino acid level, the lipase cloned from *M. bovis* Dalton 2d was shown to display the following similarity and identity to the proteins listed.

Organism	Protein	Similarity	Identity
Xenorhabdus luminescens	triacylglycerol lipase	36%	24%
Pseudomonas putida	hypothetical protein	36%	24%
Salmonella typhimurium	outer membrane esterase	35%	23%
Pseudomonas aeruginosa	lipase / esterase	36%	23%

The *M. bovis* lipase was identified as being a possible new member of the GDSL family (20) of lipolytic enzymes.

N-terminal sequencing carried out on the lipase mature protein

The required strains of *E. coli* were cultured overnight with shaking at 37°C in 500mls of luria broth. The cells were pelleted at 5,000 rpm for 15 mins and the supernatant filtered through a 0.45µm filter. Solid ammonium sulfate was added to the supernatant to 60% saturation (180g / 500ml), and dissolved at 4°C with stirring for 30 minutes. This mixture was left at 4°C overnight and the precipitated proteins pelleted at 7,000 rpm for 30 mins. The proteins were resuspended in 3ml of double distilled water and the solubilised proteins dialysed against double distilled water overnight to remove any salt. The resulting mixture was filtered through a 0.45µm filter and stored at -20°C.

Following separation of the proteins by SDS-PAGE, the proteins were transferred to PVDF membrane and excised. The protein was subjected to automated (Edman degradation) sequence analysis (28) with vapour phase delivery of critical reagents (29) in an automated sequenator (model 470A; Applied Biosystems) (Applied Biosystems Division, Foster City, CA, USA) in conjunction with a PTH amino acid separation system (model 120A PTH analyzer; Applied Biosystems).

Using this technique 17 amino acids with two gaps were identified

K E F S Q V I I F G D S L X D X G (SEQ ID NO:7)

which corresponds exactly with amino acids 26 through to 42 shown on the accompanying sequence. This result also indicated that the protein most likely includes an amino terminal signal peptide which is involved in the secretion of the protein. This amino terminal corresponds to amino acids 1 through to 25 in the accompanying sequence.

Raising antibodies to the lipase in rabbits

Antibody to the recombinant lipase was raised in rabbits by injecting ammonium sulfate precipitated supernatant from *E. coli* MC1061/pMB4. Prior to vaccination, the lipase preparation was inactivated by heating to 90°C for 90min. 30µg of this protein was injected at 2 weekly intervals for 4 weeks. The primary inoculum was emulsified with Freunds complete adjuvant and subsequent vaccinations with Freunds incomplete adjuvant.

Heat stability of *M. bovis* lipase

The recombinant lipase cloned from *M. bovis* Dalton 2d was found to be very heat stable since it required heating at 90°C for 105 minutes for the

activity to be reduced by 97%. Figure 3 illustrates this phenomenon with enzyme activity expressed as "lipase enzyme units" as determined in the extracellular lipase assay.

Relative expression levels of native versus recombinant lipase

An experiment was performed to plot growth rate with lipase production and to compare production of the recombinant lipase from MC1061/pMB1 with that of the native form of the lipase from *M. bovis* Dalton 2d. Figure 4 illustrates that the two strains grow at approximately the same rate but they do not reach the same cell density, with Dalton 2d substantially lower after 9 hours than MC1061/pMB1. Lipase expression levels were greatest from the pMB1 construct in *E. coli* compared to native lipase expression from *M. bovis* Dalton 2d.

This result was further substantiated when proteins from cell-free supernatants of either the *E. coli* clone or *M. bovis* Dalton 2d were ammonium sulfate precipitated and analysed by SDS-PAGE and western blot using antisera to the recombinant heat-deactivated lipase.

Ammonium sulfate precipitated supernatants were prepared from overnight cultures of *E. coli* or *M. bovis* that had been shaken at 37°C in either 500mls of Luria broth or brain heart infusion broth, respectively. Cells were pelleted at 5000 × g for 15 minutes and the supernatant filtered through a 0.45µm filter. Solid ammonium sulfate was added to the supernatant to 60% saturation (180g / 500ml) and dissolved at 4°C with stirring for 30 minutes. This mixture was left at 4°C overnight and the precipitated protein pelleted at 7000 × g for 30 minutes. Proteins were resuspended in 3ml of double distilled water and the solubilised proteins dialysed against double distilled water overnight to remove any salt. The resulting mixture was filtered through a 0.45µm filter and stored at -20°C.

Protein samples (100µl) were prepared for SDS-PAGE by resuspension in 100µl of 2x sample buffer (5ml 0.5M Tris (pH6.8), 8ml 10% SDS, 4ml glycerol, 0.8ml β-mercaptoethanol, 1ml double distilled H_2O, bromophenol blue) and heating to 100°C for 5 minutes. The proteins were separated on a 12.5% polyacrylamide gel using the buffer system of Laemlli (21).

Western blots were carried out according to the method of Towbin *et al.*, (22) and following separation of proteins by SDS-PAGE and transfer to nitrocellulose using the Bio-Rad minicell (Bio-Rad, Hercules, CA, USA)

transfer system. Filters were immunoblotted with the recombinant lipase antiserum (at a concentration of 1/100) which had been adsorbed against MC1061 cells. The antiserum was raised against ammonium sulfate precipitated recombinant lipase which had been heat deactivated (1 hour 45 minutes at 90°C) and used to inoculate rabbits (three doses of 50μg each) at 4 week intervals. Blood samples were collected from the marginal ear vein prior to immunisation and at each vaccination time point.

The results showed a prominent band present in the recombinant lipase positive construct MC1061/pMB1 that is detectable in relatively minor amounts in *M. bovis* Dalton 2d preparation. The protein detected with the antisera was approximately the same size as that of the predicted molecular weight for the *M. bovis* lipase (65.8kDa).

Lipase type encoded by pMB1

Thin layer chromatography (TLC) was used to determine whether the lipase of *M. bovis* Dalton 2d displayed phospholipase activity. Characterisation of phospholipase type essentially followed a previously described method (23) except that the results of separation on Silica Gel 60 plates were visualised by developing with a 10% ethanolic solution of molybdophosphoric acid at 100°C. All reagents used were purchased from Sigma (Sigma, St Louis, MO, USA).

TLC determined that the *M. bovis* lipase displayed the same enzyme specificity as that of a commercially-available phospholipase B when lysophosphatidylcholine and phosphatidylcholine were used as substrates (data not shown).

Conservation of lipase among *M. bovis*

A southern blot using an internal fragment of the Dalton 2d lipase coding region was used to investigate whether the lipase gene was present in strains of *M. bovis* representing the known pilus serotypes.

Genomic DNA extracted from the strains of *M. bovis* representing each of the known pilus serotypes (15) was digested with *Hin*dIII and separated using agarose gel electrophoresis. The DNA was transferred to a Hybond N+ filter (Amersham, Little Chalfont, Buckinghamshire, U.K.) using a previously described method (9). The probe used in the southern hybridisation was a *Hin*dIII fragment that contained sequence internal to the lipase coding

region. This fragment was labelled with α^{32}P-dATP using the Megaprime labelling system (Amersham, Little Chalfont, Buckinghamshire, U.K.) according to the manufacturers instructions. High stringency conditions were used (hybridisation temperature 68°C; 2 washes at room temperature in 2 × SSC / 0.1% SDS; 1 wash at 68°C in 0.1 × SSC / 0.1% SDS) and the resulting filters were exposed to autoradiographic film (Kodak, Rochester, New York, USA) for 5 to 24 hours before developing.

Results showed that the lipase gene is present in all strains of *M. bovis* examined.

To confirm whether or not the lipase gene was expressed in each of the serotype representative strains, antisera raised against recombinant heat deactivated lipase was used in a western blot analysis of whole cell preparations. Results showed that the lipase was indeed being expressed by all of these *M. bovis* strains.

EXAMPLE 3

Bacteria and construction of a haemolysin clone

Moraxella bovis strain Dalton 2d was a field isolate collected from a bovine eye and characterised by CSIRO Animal Health, Parkville, Australia (6).

All of the *M. bovis* strains representative of the known pilus serotypes express a haemolytic activity that is detected on horse blood agar.

Escherichia coli strain degP4::Tn5 has a leaky outer membrane and is defective in proteolysis and has been previously described (24).
All enzymes were purchased from Promega (Madison, WI, USA) except where otherwise noted.

General cloning and DNA techniques were as described (9) unless otherwise noted.

A *phoA* fusion technique that allows for the identification of exported proteins (25) was utilised with some modifications. Genomic DNA from *M. bovis* Dalton 2d (prepared using the CTAB method described in Example 1) was partially digested with *Sau*3A. Restricted DNA was ligated with a series of vectors that allow fusions with an alkaline phosphatase gene in three different reading frames. The ligated DNA was electroporated into *E. coli* degP4::Tn5 and the resulting clones screened on Luria agar containing

ampicillin (50μg/ml) and X-P (200μg/ml) (5-bromo-3-chloro-indolyl phosphate). Selection of clones relies on the observation that if the fragment is cloned in frame and contains an export sequence the resulting colony will be blue in colour. The leaky *E. coli* strain allows the outer membrane-bound proteins and secreted proteins (both fused with *phoA*) to be distinguished from non-secreted fusion proteins.

Sequencing of the *M. bovis* haemolysin determiuant

Clones selected for the presence of a secreted or outer membrane protein gene sequence were subjected to automated DNA sequencing using the methods described in Example 1. One of these clones, pMbh1, was found to contain 200bp of DNA which displayed high homology to the A subunit of other RTX toxins. Inverse PCR and degenerate oligonucleotides were utilised to obtain the sequence of the entire A subunit. The open reading frame of 2784bp was capable of encoding 928 amino acids. The sequence is written in the 5' to 3' direction and is shown in Figure 5 together with the corresponding amino acid sequence that is predicted to encode a protein with a molecular weight of 98.8kDa. The amino acid sequence is shown in SEQ. ID. NO. 5 and the DNA sequence is shown in SEQ. ID. NO. 6.

The putative start codon was identified using the RBS technique outlined above. A signal peptide analysis was not carried out as the A subunit is not secreted on its own. However as the protein sequence of these proteins (RTX) is quite highly conserved, on amino acid homologies alone this start codon was the one of choice.

Sequence homology

At the amino acid level the *M. bovis* Dalton 2d haemolysin gene product shows striking similarity to the A subunit of the of several RTX and other haemolysins as shown in the following table.

Organism	Protein	Similarity	Identity
Pasteurella haemolytica	LktA protein (leukotoxin)	68%	50%
Actinobacillus pleuropneumoniae	RTX toxin determinant	68%	48%
Escherichia coli	Haemolysin - plasmid	58%	43%
E. coli	Haemolysin - chromosomal	58%	43%

Functional complementation by the *M. bovis* haemolysin

A construct which expressed the chromosomal-borne haemolysin of *E. coli* was obtained (pLG900; generated by combining the two plasmids pLG575 (26) and pLG816 (*hlyC* and *hlyA* cloned into pBluescriptSK). pLG900 comprises the four genes of the RTX operon, *hlyC, hlyA, hlyB, hlyD,* cloned into pBluescriptSK and is capable of conferring a haemolytic phenotype on *E. coli* cells that were previously non-haemolytic. The A subunit (*hlyA*) of this construct was mutated such that it was no longer able to be expressed but the other genes involved in the operon (*hlyB, hlyC* and *hlyD*) remained intact. The *E. coli* strain containing this construct (pLG900 / *hlyA* negative) was no longer haemolytic. However, the haemolytic phenotype was restored by providing *in trans* the cloned haemolysin subunit gene from *M. bovis* Dalton 2d. Thus it was confirmed that the cloned *M. bovis* haemolysin gene encoded a structural subunit that was most probably a member of the RTX family of haemolytic enzymes.

Further sequence analysis has established that, like other members of the family, the *M. bovis* RTX A subunit gene is flanked by DNA sequences capable of encoding the RTX B,C and D proteins.

Conservation of the RTX A subunit among *M. bovis*

To determine whether the gene for the RTX A subunit was present in *M. bovis* strains representing the known pilus serotypes, a southern hybridisation analysis was performed using the coding region of the RTX A subunit as a probe.

Genomic DNA extracted from the seven serotype strains of *M. bovis* (15) was digested with *Eco*RV and separated using agarose gel electrophoresis. The DNA was transferred to a Hybond N+ filter (Amersham,

Little Chalfont, Buckinghamshire, U.K.) using a previously described method (9). The probe used was a PCR amplified product that contained all of the coding region from the A subunit of the RTX haemolysin of *M. bovis*. This fragment was labelled with α^{32}P-dATP using the Megaprime labelling system (Amersham, Little Chalfont, Buckinghamshire, U.K.) according to the manufacturers instructions. High stringency conditions were used (hybridisation temperature 68°C; 2 washes at room temperature in 2 × SSC / 0.1% SDS; 1 wash at 68°C in 0.1 × SSC / 0.1% SDS) and the resulting filters were exposed to autoradiographic film (Kodak, Rochester, New York, USA) for 5 to 24 hours before developing.

Results showed that the gene encoding the RTX A haemolysin subunit was conserved in all seven representative strains of *M. bovis* examined. Interestingly, each of these strains is known to display the haemolytic phenotype on horse blood agar. In contrast, the non-haemolytic *M. bovis* strain Gordon 26L3 did not hybridise to the RTX A gene probe possibly suggesting that *M. bovis* contains only a single structural gene responsible for the haemolytic phenotype detected on horse blood agar.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

References

1. Moore L. J., and A. W. D. Lepper. (1991). Vet Microbiol. 29:75-83.

2. Lehr, C. H., G. Jayappa and R. A. Goodnow. (1985). Cornell Vet. 75:484-492.

3. Pugh, G. W., D. E. Hughes and G. D. Booth. (1977). Am J Vet Res. 38:1519-1522.

4. Frank, S. K., and J. D. Gerber. (1981). J Clin Microbiol. 13(2):269-271.

5. Billson, F.M., J. L. Hodgson, A. W. Lepper, W. P. Michalski, C. L. Schwartzkoff, P. R. Lehrbach, and J. M. Tennent. (1994). FEMS Microbiol Lett. 124(1):69-73.

6. Elleman, T. C., P. A. Hoyne, and A. W. D. Lepper. (1990). Infect Immun. 58(6):1678-1684.

7. Woodcock, D. M., P. J. Crowther, J. Doherty, S. Jefferson, E. DeCruz, M. Noyer-Weidner, S. S. Smith, M. Z. Michael, and M. W. Graham. (1989). Nucleic Acids Res. 17(9):3469-3478.

8. Raleigh, E. A., K. Lech, and R. Brent. (1989). In Current Protocols in Molecular Biology eds. Ausubel, F. M. et al., Publishing Associates and Wiley Interscience; New York. Unit 1.4.

9. Sambrook, J., E. F. Fritsch, and T. Maniatis. (1989). Molecular Cloning: A Laboratory Manual, 2nd edition. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York.

10. Dillela, A. G., and S. L. C. Woo. (1985). Focus. 7(2):1-5.

11. Hohn, B., and J. Collins. (1980). Gene. 11(3-4):291-298.

12. Narberhaus. F., K. Giebeler, and H. Bahl. (1992). J Bacteriol. 174(10):3290-3299.

13. Altschul, S. F., T. L. Madden, A. A. Schaffer, Z. Jinghui, Z. Zhang, W. Miller, and D. J. Lipman. (1997). Nucleic Acids Res. 25:3389-3402.

14. Bourgeau, G., H. Lapointe, P. Peloquin, and D. Mayrand. (1992). Infection and Immunity. 60(8):3186-3192.

15. Ausubel, F. M. R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. (1994). Current Protocols in Molecular Biology. Green Publishing Associates, Inc. and John Wiley and Sons Inc. New York.

16. Wertman, K. F., A. R. Wyman, and D. Botstein. (1986). Gene. 49(2):253-262.

17. Bolivar F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heynecker, and H. W. Boyer. (1977). Gene. 2(2):95-113.

18. Stuer, W., K. E. Jaeger, and U. K. Winkler. (1986). J Bacteriol. 168:1070-1074.

19. Winkler, U. K., and M. Stuckman. (1979). J Bacteriol. 138:663-670.

20. Upton, C., and J. T. Buckley. (1995). Trends Biochem Sci. 20(5):178-9.

21. Laemmli, U. K. (1970). Nature. 227(259):680-685.

22. Towbin, H., T. Staehlin, and J. Gordon. (1979). Proc Natl Acad Sci USA. 76(9):4350-4354.

23. Fifis, T., C. Costopoulos, and J. A. Vaughn. (1996). Vet Microbiol. 49:219-233.

24. Strauch, K. L., and J. Beckwith. (1988). Proc Natl Acad Sci USA. 85(5):1576-1580.

25. Gilaldi, M., C. I. Champion, D. A. Haake, D. R. Blanco, J. F. Miller, J. N. Miller, and M. A. Lovett. (1993). J Bacteriol. 175:4129-4136.

26. Mackman, N., J-M. Nicaud, L. Gray, and I. B. Holland. (1985). Mol Genet. 201:282-288.

27. Atwell, J. L., J. M. Tennent, A. W. Lepper and T. C. Elleman. (1994). J Bacteriol 176(16):4875-82 .

28. Edman, P., and C. Begg. (1967). Eur. J. Biochem. 1:80-91.

29. Hewick, R. M., M. W. Hunkapillar, L. E. Hood, and W. J. Dreyer. 1981. J Biol Chem. 256: 7990-7997

Claims:

1. A polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO. 1 from amino acid 37 to 1114, or a sequence having at least 50% identity thereto, or a functional fragment thereof.

2. A polypeptide as claimed in claim 1, the polypeptide having a sequence of at least 70% identity with the sequence shown in SEQ. ID. NO:1 from amino acid 37 to 1114.

3. A polypeptide as claimed in claim 1, the polypeptide having a sequence of at least 80% identity with the sequence shown in SEQ. ID. NO:1 from amino acid 37 to 1114.

4. A polypeptide as claimed in claim 1, the polypeptide having a sequence of at least 90% identity with the sequence shown in SEQ. ID. NO:1 from amino acid 37 to 1114.

5. A polypeptide as claimed in any one of claims 1 to 4, the polypeptide having protease activity.

6. A nucleic acid molecule, the nucleic acid molecule comprising a sequence encoding a polypeptide as claimed in any one of claims 1 to 5.

7. A nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO:2 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

8. A nucleic acid molecule as claimed in claim 7, the nucleic acid molecule comprising a sequence having least 70% identity with the sequence shown in SEQ. ID. NO:2.

9. A nucleic acid molecule as claimed in claim 7, the nucleic acid molecule comprising a sequence having least 80% identity with the sequence shown in SEQ. ID. NO:2.

10. A nucleic acid molecule as claimed in claim 7, the nucleic acid molecule comprising a sequence having least 90% identity with the sequence shown in SEQ. ID. NO:2.

11. A composition for use in raising an immune response in an animal, the composition comprising the polypeptide as claimed in any one of claims 1 to 5 or a nucleic acid sequence as claimed in claim 6 and optionally a carrier and/or adjuvant.

12. A polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO:3 from amino acid 26 to 616, or a sequence having at least 50% identity thereto, or a functional fragment thereof.

13. A polypeptide as claimed in claim 12, the polypeptide having a sequence of at least 70% identity with the sequence shown in SEQ. ID. NO:3 from amino acid 26 to 616.

14. A polypeptide as claimed in claim 12, the polypeptide having a sequence of at least 80% identity with the sequence shown in SEQ. ID. NO:3 from amino acid 26 to 616.

15. A polypeptide as claimed in claim 12, the polypeptide having a sequence of at least 90% identity with the sequence shown in SEQ. ID. NO:3 from amino acid 26 to 616.

16. A polypeptide as claimed in any one of claims 12 to 15, the polypeptide having lipase activity.

17. A nucleic acid molecule, the nucleic acid molecule comprising a sequence encoding a polypeptide of any one of claims 12 to 16.

18. A nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO:4 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

19. A nucleic acid molecule as claimed in claim 18, the nucleic acid molecule comprising a sequence having at least 70% identity with the sequence shown in SEQ. ID. NO:4.

20. A nucleic acid molecule as claimed in claim 18, the nucleic acid molecule comprising a sequence having at least 80% identity with the sequence shown in SEQ. ID. NO:4.

21. A nucleic acid molecule as claimed in claim 18, the nucleic acid molecule comprising a sequence having at least 90% identity with the sequence shown in SEQ. ID. NO:4.

22. A composition for use in raising an immune response in an animal, the composition comprising a polypeptide as claimed in any one of claims 12 to 16 or a nucleic acid sequence as claimed in claim 17 and optionally a carrier and/or adjuvant.

23. A polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO:5, or a sequence having at least 60% identity thereto, or a functional fragment thereof.

24. A polypeptide as claimed in claim 23, the polypeptide having a sequence of at least 70% identity with the sequence shown in SEQ. ID. NO:5.

25. A polypeptide as claimed in claim 23, the polypeptide having a sequence of at least 80% identity with the sequence shown in SEQ. ID. NO:5.

26. A polypeptide as claimed in claim 23, the polypeptide having a sequence of at least 90% identity with the sequence shown in SEQ. ID. NO:5.

27. A polypeptide as claimed in any one of claims 23 to 26, the polypeptide having haemolysin activity.

28. A nucleic acid molecule, the nucleic acid molecule comprising a sequence encoding a polypeptide of any one of claims 23 to 27.

29. A nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO:6 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

30. A nucleic acid molecule as claimed in claim 29, the nucleic acid molecule comprising a sequence having at least 70 % identity with the sequence shown in SEQ. ID. NO:6.

31. A nucleic acid molecule as claimed in claim 29, the nucleic acid molecule comprising a sequence having at least 80 % identity with the sequence shown in SEQ. ID. NO:6.

32. A nucleic acid molecule as claimed in claim 29, the nucleic acid molecule comprising a sequence having at least 90 % identity with the sequence shown in SEQ. ID. NO:6.

33. A composition for use in raising an immune response in an animal, the composition comprising a polypeptide of any one of claims 23 to 27 or a nucleic acid sequence of claim 28 and optionally a carrier and/or adjuvant.

34. A composition for use in raising an immune response in an animal directed against *Moraxella*, the composition comprising at least one polypeptide selected from the group consisting of a polypeptide as claimed in any one of claims 1 to 5, a polypeptide as claimed in any one of claims 12 to 16, and a polypeptide as claimed in any one of claims 23 to 27, and optionally including an adjuvant or carrier.

35. A composition as claimed in claim 34, the composition comprising a polypeptide as claimed in any one of claims 23 to 27 and either one of, or preferably both of, a polypeptide as claimed in any one of claims 1 to 5 and a polypeptide as claimed in any one of claims 12 to 16.

36. A composition as claimed in claim 34 or claim 35 wherein the *Moraxella* is *M. bovis or M. catarrhalis*.

37. A composition as claimed in claim 34 or claim 35 wherein the *Moraxella* is *M. bovis*.

38. An antibody raised against a polypeptide selected from the group consisting of a polypeptide as claimed in any one of claims 1 to 5, a polypeptide as claimed in any one of claims 12 to 16, and a polypeptide as claimed in any one of claims 23 to 27.

```
1     ttctcatgtt tgacagctta tcatcgataa gctttaatgc ggtagtttat cacagttaaa ttgctaacgc agtcaggcac cgtgtatgaa

91    atctaacaat gcgctcatcg tcatcctcgg caccgtcacc ctggatgctg taggcatagg cttggttatg ccggtactgc cgggcctctt

181   gcgggatatc gtccattccg acagcatcgc cagtcactat ggcgtgctgc tagcgctata tgcgttgatg caatttctat gcgcacccgt

271   tctcggagca ctgtccgacc gctttggccg ccgcccagtc ctgctcgctt cgctacttgg agccactatc gactacgcga tcatggcgac

361   cacaccgtc ctgtggatca ataattaatg aacatatata ctctatttaa tatttcttat ttattcgtaa tattgccata aaaataatac

451   attatttcta tattaactaa actgttaata tttgtaaata ataaacattt gtttatctaa aaaaataaat aatataaatc aagcaattac

541   aatcttattt ttgaaaatac aataatactg caattgctta atctagacat taagtttatt tttgattaaa attgccaaaa cttgtgtaaa

631   taagtttcac cgaattgata ctttaagggt atcaatattg caacatggta aatgattact atgttgttgg gcattgcata aattgtctat

721   aataacttgt tatggatgat tgatggcaat gataaactta gtgacaatga taaacgcaaa gaggtgtaat atgtcattac aaactcaacc
1                                                                                 M  S  L  Q  T  Q

811   tgccaagaga gggttctatg ttaagcattt aagtatggct tgcatgctgg taattagtgc tagtagtacg gtaagttatg ccaactcagc
7     P  A  K  R  G  F  Y  V  K  P  L  S  M  A  C  M  L  V  I  S  A  S  S  T  V  S  Y  A  N  S

901   tccaatgatt gttgattcac agtacaatag ttctaaatac tctttctacg attactattt agatttcctt aaacgtttta gaccaactcc
37    A  P  M  I  V  D  S  Q  Y  N  S  S  K  Y  S  F  Y  D  Y  Y  L  D  F  L  K  R  F  R  P  T

991   aactccagtg ccaagccctg tgagaccggc tcctgaactc gttcgtccga ccccagcccc gattccggct ccaacgcctg tgccaacacc
67    P  T  P  V  P  S  P  V  R  P  A  P  E  L  V  R  P  T  P  A  P  I  P  A  P  T  P  V  P  T

1081  ggcaccaatt agtggcggta tatcaggtag ctatattgct ccagtatcgc catcagaggt gagacagcct gattacacaa gacgcgttca
97    P  A  P  I  S  G  G  I  S  G  S  Y  I  A  P  V  S  P  S  E  V  R  Q  P  D  Y  T  R  R  V

1171  agccaatcta aaacgcaacc aacctgcacc aagtgctggc acacgtacag gttatagtgt catggatacg tcaaataatt ctaatttgac
127   Q  A  N  L  K  R  N  Q  P  A  P  S  A  G  T  R  T  G  Y  S  V  M  D  I  S  N  N  S  N  L

1261  atctaaattt tatggcacaa ccgaagatgg ttatgccgag cgtcttgaca acctaaagaa caccattgat acacgtcaag ccaaagtagg
157   T  S  K  F  Y  G  T  T  E  D  G  Y  A  E  R  L  D  N  L  K  N  T  I  D  T  R  Q  A  K  V

1351  tgtgattgat acaggcatta accgcttcaa ccgagacttg gttggtgcaa atgtgcatga tacacagatt gagtgtgttt ctgctggacg
187   G  V  I  D  T  G  I  N  R  F  N  R  D  L  V  G  A  N  V  H  D  T  Q  I  E  C  V  S  A  G

1441  ttccacctgc tatacgccag aaaatgattc aggcattgtt gaaatcccaa caacctctgc tagtggtagt catggcaacc aaatggcggc
217   R  S  T  C  Y  T  P  E  N  D  S  G  I  V  E  I  P  T  T  S  A  S  G  S  H  G  N  Q  M  A

1531  tgtcatcgct ggtaacaacg gcatgaccaa cgccaaaatt tacggcagtg acagtattga tcgacgttca aatggtggca accatttctt
247   A  V  I  A  G  N  N  G  M  T  N  A  K  I  Y  G  S  D  S  I  D  R  R  S  N  G  G  N  H  F

1621  gatgatgcgt aagctgaacc aagaccatgg tgtcaagatt tttaacaact cttggggttc taacaacact gaccaatggt actacgatgc
277   L  M  M  R  K  L  N  Q  D  H  G  V  K  I  F  N  N  S  W  G  S  N  N  T  D  Q  W  Y  Y  D

1711  tcagcgccta aattacaatc ctactacagg acagattaat ccaaatcctt acagaaccag tattaccaat gctgaagtga ctttgcctgt
307   A  Q  R  L  N  Y  N  P  T  T  G  Q  I  N  P  N  P  Y  R  T  S  I  T  N  A  E  V  T  L  P

1801  cattcatgat cttattatga atcgtgactc gcttatcatt aaagcaacag gtaacgaagg cttgaacgat gctcatgatg aaaacctagc
337   V  I  H  D  L  I  H  N  R  D  S  L  I  I  K  A  T  G  N  E  G  L  N  D  A  H  D  E  N  L

1891  accgctcatg aacagcaact tcaaaaaagg tttcattact gtctcctcgc ctagagaaga ttttggtaaa gcgaatcatt gtggtcgaac
367   A  P  L  M  N  S  N  F  K  K  G  F  I  T  V  S  S  P  R  E  D  F  G  K  A  N  H  C  G  R

1981  tgccgaatgg tgtgtatccg caacatcatc tacccaaaat tacgccaacg atggcagact gagtagctat aagggtacat cacctgcaac
397   T  A  E  W  C  V  S  A  T  S  S  T  Q  N  Y  A  N  D  G  R  L  S  S  Y  K  G  T  S  P  A

2071  cgctcgtgtg tccggcacgg cagtgctcgt gcaatctgct tatccttgga tgaaaaatga aaatatctct caaaccattt tgggtactgc
427   T  A  R  V  S  G  T  A  V  L  V  Q  S  A  Y  P  W  M  K  N  E  N  I  S  Q  T  I  L  G  T

2161  caaggatttc tcagagatta ctgccaattc acctaatggc taccaaggac taagaaaggt tagtagattg ccatctggtt attacggctc
457   A  K  D  F  S  E  I  T  A  N  S  P  N  G  Y  Q  G  L  R  K  V  S  R  L  P  S  G  Y  Y  G

2251  ttattacact gacaatcagg gtaatttcta tgttcctggc aatgtcaatt gggaaaaccg tcgaattgtc gctaatcata acggcaagaa
487   S  Y  Y  T  D  N  Q  G  N  F  Y  V  P  G  N  V  N  W  E  N  R  R  I  V  A  N  H  N  G  K

2341  cattacatgg gaagatggtt ggggttttgtt agatccagaa gcggccgcta aaggttatgg tggtttctat tgggataatg tggaattaga
517   N  I  T  W  E  D  G  W  G  L  L  D  P  E  A  A  A  K  G  Y  G  G  F  Y  W  D  N  V  E  L

2431  cactaaaggc acgcctttat ctgtattcta caatgaccta aaaggtgata aaggctttac caaaaaaggt gaaggtaaac ttgtctttac
547   D  T  K  G  T  P  L  S  V  F  Y  N  D  L  K  G  D  K  G  F  T  K  K  G  E  G  K  L  V  F

2521  tggtaataat agctataaaq gcgactctgt catcgagggt ggttcactag aagtaaatgg taacaacggt ggttcaacca tggttgttaa
577   T  G  N  N  S  Y  K  G  D  S  V  I  E  G  G  S  L  E  V  N  G  N  N  G  G  S  T  M  V  V

2611  aggtggtgaa ctaacaggtt atggtaatgt agctaatgtt cgtcaaacag gtggttgggt taacaacgaa ggtaacctaa acatcagagg
607   K  G  G  E  L  T  G  Y  G  N  V  A  N  V  R  Q  T  G  G  W  V  N  N  E  G  N  L  N  I  R
```

Figure 1

```
2701  tgactacaac atcaacactc aacgtggcgt ggatgctggt ctaaaagctc aatttggcaa catgcttacc gtggacggta aggccaaact
637   G  D  Y  N  I  N  T  Q  R  G  V  D  A  G  L  K  A  Q  F  G  N  M  L  T  V  D  G  K  A  K

2791  aggtggtaca ctaaatctaa ctggtgagac caaagatggt atcatcagca aatcaggtag ccgtagcact gtacttcgtg ctaagcgtgg
667   L  G  G  T  L  N  L  T  G  E  T  K  D  G  I  I  S  K  S  G  S  R  S  T  V  L  R  A  K  R

2881  tcttgaaggt caatttgaca attatcgttc aagcaaccca ttatttgaag taacaaatgt tgaatatacg ccagaagtag acagaaatgg
697   G  L  E  G  Q  F  D  N  Y  R  S  S  N  P  L  F  E  V  T  N  V  E  Y  T  P  E  V  D  R  N

2971  cagagtggta ggtggttcac gcacgaacaa tgacgtgcaa gtaactgcca aacgtctaag tgcaggcaat gttgtttatg gcatcagcat
727   G  R  V  V  G  G  S  R  T  N  N  D  V  Q  V  T  A  K  R  L  S  A  G  N  V  V  Y  G  I  S

3061  gaatgacagt ggtagccgtg ttgcacaaaa cctagacaaa gtacttaatg atttagataa aaaacaagaa acacaaggtt cactgaccag
757   M  N  D  S  G  S  R  V  A  Q  N  L  D  K  V  L  N  D  L  D  K  K  Q  E  T  Q  G  S  L  T

3151  tgatgagaag caatttgcta accgtgtatt cactggtttt gaaaacatga attctggtgc agaatctaaa ctttctacag taagcaccaa
787   S  D  E  K  Q  F  A  N  R  V  F  T  G  F  E  N  M  N  S  G  A  E  S  K  L  S  T  V  S  T

3241  ccgtgagcta tacaagcttg acccaacttt ctatgctgac agtgcattaa acgcagtaga agacagtgct aaccatgcaa ccgaatttgg
817   N  R  E  L  Y  K  L  D  P  T  F  Y  A  D  S  A  L  N  A  V  E  D  S  A  N  H  A  T  E  F

3331  taagcgtgtt agcgccccaa gaggtgtttg gggtaatatc agtcaccatg attatgatgt agaactagag catgctacaa gtgcacgtaa
847   G  K  R  V  S  A  P  R  G  V  W  G  N  I  S  H  H  D  Y  D  V  E  L  E  H  A  T  S  A  R

3421  aggcaacaac attagtgttg gtgcaagcac tcaaactgca gccgacatta gtgttggtgc acaacttgat gtaagtaaac ttgacttgga
877   K  G  N  N  I  S  V  G  A  S  T  Q  T  A  A  D  I  S  V  G  A  Q  L  D  V  S  K  L  D  L

3511  agaatctgtt tatggtattg gcaacaaaac caaaactgac agcattggct tgactgttgg tgcttctaag aagttgggtg atgcctatct
907   E  E  S  V  Y  G  I  G  N  K  T  K  T  D  S  I  G  L  T  V  G  A  S  K  K  L  G  D  A  Y

3601  atcaggttgg gtaaaaggtg ccaaagttga tacagaagcg aaccgtgggg aaaactctaa caaagttgag tacaatggta agctatatgg
937   L  S  G  W  V  K  G  A  K  V  D  T  E  A  N  R  G  E  N  S  N  K  V  E  Y  N  G  K  L  Y

3691  tgctggtatc caagcgggta caaacattga tactgcatcg ggcgtgagtg tacaacctta tgcctttgtt aaccatcagc agtacaaaaa
967   G  A  G  I  Q  A  G  T  N  I  D  T  A  S  G  V  S  V  Q  P  Y  A  F  V  N  H  Q  Q  Y  K

3781  cgatggtagc ttcaatgacg gtcttaacgt tgttgacgac atcgaagcaa aacaaactca ggtaggtgtg ggtgctgata tggtgttcca
997   N  D  G  S  F  N  D  G  L  N  V  V  D  D  I  E  A  K  Q  T  Q  V  G  V  G  A  D  H  V  F

3871  agcaacacct gctctacagc ttactggtgg tgtgcaagtt gctcacgctg ttagccgtga caccaaccta gacactcgct atgttggtac
1027  Q  A  T  P  A  L  Q  L  T  G  G  V  Q  V  A  H  A  V  S  R  D  T  N  L  D  T  R  Y  V  G

3961  agcgacagat gtacagtatg gcacttggga tactgacaaa accaaatggt cagccaaggt tggtgctaac tataatgtga caccaaacag
1057  T  A  T  D  V  Q  Y  G  T  W  D  T  D  K  T  K  W  S  A  K  V  G  A  N  Y  N  V  T  P  N

4051  ccaagtgggt ctaaactaca gctacacagg tagtggcgat tcagatgctt cccaagtggg tgtgagcttc accagcaagt tctaattcat
1087  S  Q  V  G  L  N  Y  S  Y  T  G  S  G  D  S  D  A  S  Q  V  G  V  S  F  T  S  K  F  -

4141  taataaggca acaaaaaaca gcacaatttc ggttgtgctg ttttttgtga tgccgagcgt aaaatttccc caaaaaaagc gtgataatta

4231  ccacgctttt ttattgcata ttgcaaaata gtattgcatt tatgggttgt taagcaaccc gtccaaatac cccctaaaca actccacccc

4321  aatcggtgct aacttgtttt gccacaggct cgtcaatgtg tcggcatcat caaccattac cgac
```

Figure 1 (continued)

```
   1  tgggcagata acccatcaaa gacccaaagc aacccataaa tcaaaaaaac acttgtaatt tgtgtaatat cttgttacac tttacaagtg

  91  ttttttacttt gaaagcaact cagaagtaa taatgaaaaa atccgccttt gccaaatact cagcacttgc cctaatggtt gggatgtgcc
   1                                  M  K  K  S  A  F  A  K  Y  S  A  L  A  L  M  V  G  M  C

 181  tgcacaccgc ttacgccaag gagtttagcc aagtcatcat ttttggggac agcttgtccg atacaggtcg cctaaaagat atggtcgccc
  20  L  H  T  A  Y  A  K  E  F  S  Q  V  I  I  F  G  D  S  L  S  D  T  G  R  L  K  D  M  V  A

 271  gaaaagatgg caccgttggc aacaccttac agccatcttt taccaccaac cccgaccctg tatggtcaag cttatttgcc caaagttatg
  50  R  K  D  G  T  L  G  N  T  L  Q  P  S  F  T  T  N  P  D  P  V  W  S  S  L  F  A  Q  S  Y

 361  gcaaaaccgc cagtgccaac acgccctaca atcccactgg cactaactat gccgtgggcg gagctcgctc tggctcggag gtcaattgga
  80  G  K  T  A  S  A  N  T  P  Y  N  P  T  G  T  N  Y  A  V  G  G  A  R  S  G  S  E  V  N  W

 451  atggttttgt gaatgtaccc tccaccaaaa cgcaaatcac cgaccatttg accgccacag gtggcaaagc cgaccctaat accctgtatg
 110  N  G  F  V  N  V  P  S  T  K  T  Q  I  T  D  H  L  T  A  T  G  G  K  A  D  P  N  T  L  Y

 541  ccatttggat tggctctaat gacttaattt cagcttctca agccaccaca acagccgaag cccaaaacgc cattaaaggt gcggtaactc
 140  A  I  W  I  G  S  N  D  L  I  S  A  S  Q  A  T  T  T  A  E  A  Q  N  A  I  K  G  A  V  T

 631  gcaccgtgat agacatcgaa acactcaatc aagcaggggc gacaaccatt ttggtgccaa atgtgcctga tttgagcctc acgccccgag
 170  R  T  V  I  D  I  E  T  L  N  Q  A  G  A  T  T  I  L  V  P  N  V  P  D  L  S  L  T  P  R

 721  ccatctatgg cgaaagcctc atggcaggcg tgcaagacaa agccaaactc gcctcaagtc tgtataatag cggtctgttt gaagcattaa
 200  A  I  Y  G  E  S  L  M  A  G  V  Q  D  K  A  K  L  A  S  S  L  Y  N  S  G  L  F  E  A  L

 811  atcaatccac cgccaacatc atccctgcca acacctttgc cctactccaa gaagcgacca caaataaaga agcctttggt tttaaaaaca
 230  N  Q  S  T  A  N  I  I  P  A  N  T  F  A  L  L  Q  E  A  T  T  N  K  E  A  F  G  F  K  N

 901  cgcaaggcgt ggcgtgtcaa atgcccgctc gtaccacagg ggcggatgat gtggcttcta cttccttggc atgtaccaaa gccaatctta
 260  T  Q  G  V  A  C  Q  M  P  A  R  T  T  G  A  D  D  V  A  S  T  S  L  A  C  T  K  A  N  L

 991  tagaaaacgg ggcaaatgac acctacgcct ttgccgatga cattcaccca tcgggacgca cgcaccgcat tttggcacag tattaccgtt
 290  I  E  N  G  A  N  D  T  Y  A  F  A  D  D  I  H  P  S  G  R  T  H  R  I  L  A  Q  Y  Y  R

1081  ctatcatgga cgcccctact cacatgggta aacttctcagg cgagcttgtc aaaacaggtt cagcccacga ccgtcatgtt taccgtcagc
 320  S  I  M  D  A  P  T  H  M  G  K  L  S  G  E  L  V  K  T  G  S  A  H  D  R  H  V  Y  R  Q

1171  ttgacaggct tagtggctca cagcacagca tttgggcaaa cgtccatgcc agcgaccgta ccgaccccac cacccaaatc ggcttggacg
 350  L  D  R  L  S  G  S  Q  H  S  I  W  A  N  V  H  A  S  D  R  T  D  P  T  T  Q  I  G  L  D

1261  tggcaggttc atcaagccat acaggggcgt atctgagcca ccaaaaccaa gattatgtgc tggatgacac cctatcatca gatgtcaaaa
 380  V  A  G  S  S  S  H  T  G  A  Y  L  S  H  Q  N  Q  D  Y  V  L  D  D  T  L  S  S  D  V  K

1351  ccattggcat ggggctgtat catcgccatg acatcggcaa tgtccgtcta aaaggcgtgg caggtatcga ccgacttagc gtggatacgc
 410  T  I  G  M  G  L  Y  M  R  H  D  I  G  N  V  R  L  K  G  V  A  G  I  D  R  L  S  V  D  T

1441  accgccatat cgactgggag ggggcaagcc gttcgcacac ggcagacacc accgccagac gttttcatgc agggctacaa gccagcctatg
 440  H  R  H  I  D  W  E  G  A  S  R  S  H  T  A  D  T  T  A  R  R  F  H  A  G  L  Q  A  S  Y

1531  gcatagacat gggcaaagcc accgtgcgtc cgcttatcgg cgtacatgcc caaaaagtca aagtgcgtga tttggtagag aatgagccta
 470  G  I  D  M  G  K  A  T  V  R  P  L  I  G  V  H  A  Q  K  V  K  V  R  D  L  V  E  N  E  P

1621  ccctatccac cgccatgcgt tttggcgagc aagaacaaaa gtccctacaa ggcgagattg gcgtcgatgt ggcttatccg attagccctg
 500  T  L  S  T  A  M  R  F  G  E  Q  E  Q  K  S  L  Q  G  E  I  G  V  D  V  A  Y  P  I  S  P

1711  ctttgactct gacgggcggt atcgctcacg ctcatgagtt taacgatgat gaacgcacca ttaatgccac tttaacctcc attcgtgaat
 530  A  L  T  L  T  G  G  I  A  H  A  H  E  F  N  D  D  E  R  T  I  N  A  T  L  T  S  I  R  E

1801  acacgaaggg ctttaataca agcgttagca ccgacaaatc tcacgccacc accgctcatc tgggcgtaca agggcaactt ggcaaggcaa
 560  Y  T  K  G  F  N  T  S  V  S  T  D  K  S  H  A  T  T  A  H  L  G  V  Q  G  Q  L  G  K  A

1891  atattcatgc aggcgttcac gccacccacc aagacagcga tacagacgtg ggtggttcgc ttggggttcg cttgatgttt tgattggctt
 590  N  I  H  A  G  V  H  A  T  H  Q  D  S  D  T  D  V  G  G  S  L  G  V  R  L  M  F  -

1981  ttaaagataa aaagtggtat catgccactt tttattttgc caaaaatcta tgtttgagta catcaaagcc tttcacatca tcgccatgcg

2071  atgataagct gtcaaacatg ag
```

Figure 2

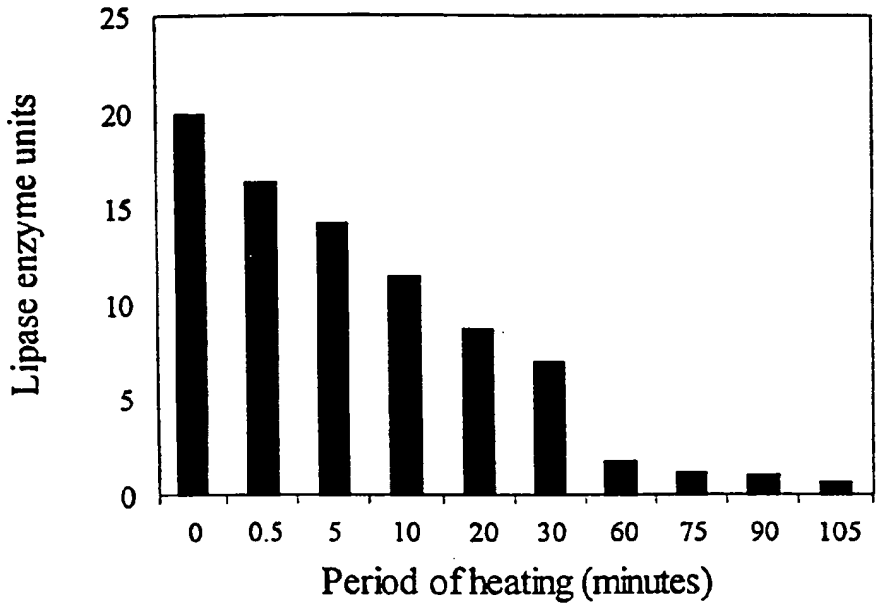
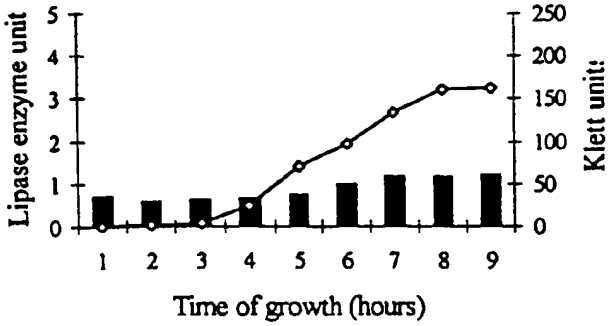

25
20
15
10
5
0
Lipase enzyme units
0
0.5
5
10
20
30
60
75
90
105
Period of heating (minutes)

Figure 3

(i) Dalton 2d / *M. bovis*



(ii) pMB1 / MC1061

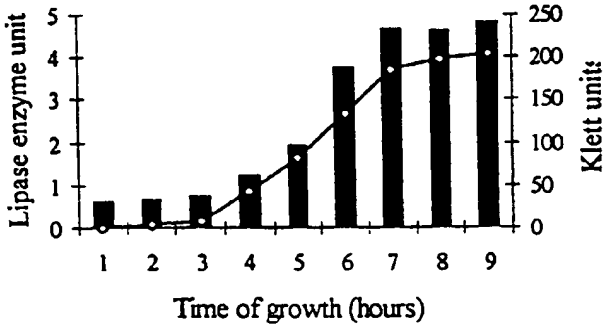


Figure 4

```
1    atgagaacgt tattttcaga tgaattgttt agagcgattc gtgtagatgg aaattcatcg catggtaaga tatctgaatt ttatggaaag
       M  R  T  L  F  S  D  E  L  F  R  A  I  R  V  D  G  N  S  S  H  G  K  I  S  E  F  Y  G  K

91   tctgttgatt caaaattagc ctcaagaata tttgcacaat atcacgaaga tttgacgagc aaattgtcaa ctcagaataa ttttattata
       S  V  D  S  K  L  A  S  R  I  F  A  Q  Y  H  E  D  L  T  S  K  L  S  T  Q  N  N  F  I  I

181  tctaaagata attaatacaa ccttttctaa cacaacgagg agaaacatat tatgtccaat ataaatgtaa ttaaatctaa tattcaagca
1      S  K  D  N  -                                                  M  S  N  I  N  V  I  K  S  N  I  Q  A

271  ggcttgaatt caacaaagtc tggattaaaa aatctttact tggctattcc caaagattat gatccgcaaa aaggtgggac tttaaatgat
14     G  L  N  S  T  K  S  G  L  K  N  L  Y  L  A  I  P  K  D  Y  D  P  Q  K  G  G  T  L  N  D

361  tttattaaag ctgctgatga attaggtatt gctcgtttag cagaagagcc taatcacact gaaacagcaa aaaaatctgt tgacacagta
44     F  I  K  A  A  D  E  L  G  I  A  R  L  A  E  E  P  N  H  T  E  T  A  K  K  S  V  D  T  V

451  aatcagtttc tctctctcac acaaactggt attgctattt ctgcaacaaa attagaaaag ttcttacaaa aacattctac caataagtta
74     N  Q  F  L  S  L  T  Q  T  G  I  A  I  S  A  T  K  L  E  K  F  L  Q  K  H  S  T  N  K  L

541  gccaaagggt tagacagtgt agaaaatatt gatcgtaaat taggtaaagc aagtaatgta ttatcaacat taagctcttt tttgggcact
104    A  K  G  L  D  S  V  E  N  I  D  R  K  L  G  K  A  S  N  V  L  S  T  L  S  S  F  L  G  T

631  gcattagcgg gtatagaact tgattcttta atcaaaaaag gtgatgctgc acctgatgct ttggctaaag ctagtattga cttgattaat
134    A  L  A  G  I  E  L  D  S  L  I  K  K  G  D  A  A  P  D  A  L  A  K  A  S  I  D  L  I  N

721  gagataattg gtaatctatc tcagagtact caaacgattg aagcattttc ttcacagtta gcaaagttag gttctactat atcgcaggct
164    E  I  I  G  N  L  S  Q  S  T  Q  T  I  E  A  F  S  S  Q  L  A  K  L  G  S  T  I  S  Q  A

811  aaaggcttct ctaatatagg aaacaagttg caaaacttaa atttttctaa aacaaatctt ggtttggaaa taattactgg tttgctatca
194    K  G  F  S  N  I  G  N  K  L  Q  N  L  N  F  S  K  T  N  L  G  L  E  I  I  T  G  L  L  S

901  ggcatttctg caggctttgc tttagcggat aaaaatgcat cgactggcaa aaaagttgct gcaggttttg aattaagcaa tcaagttatt
224    G  I  S  A  G  F  A  L  A  D  K  N  A  S  T  G  K  K  V  A  A  G  F  E  L  S  N  Q  V  I

991  ggtaatgtaa caaaagcaat ttcttcatat gttttagcac aacgtgttgc tgctggtcta tcaactactg gtgctgttgc tgctttaatt
254    G  N  V  T  K  A  I  S  S  Y  V  L  A  Q  R  V  A  A  G  L  S  T  T  G  A  V  A  A  L  I

1081 acttcatcga ttatgttggc aattagtcct ttggcattta tgaatgcagc agataaattc aatcatgcta atgctcttga tgagtttgca
284    T  S  S  I  M  L  A  I  S  P  L  A  F  M  N  A  A  D  K  F  N  H  A  N  A  L  D  E  F  A

1171 aaacaattcc gaaaatttgg ctatgatggg gatcatttat tggctgaata tcagcgtggt gtgggtacta ttgaagcttc attaactaca
314    K  Q  F  R  K  F  G  Y  D  G  D  H  L  L  A  E  Y  Q  R  G  V  G  T  I  E  A  S  L  T  T

1261 attagtacgg cattaggtgc agtttctgct ggtgtttccg ctgctgctgt aggatctgct gttggtacac cgattgcact attagttgca
344    I  S  T  A  L  G  A  V  S  A  G  V  S  A  A  A  V  G  S  A  V  G  T  P  I  A  L  L  V  A

1351 ggtgttacag gattgatctc tggaatttta gaagcgtcta aacaggcaat gtttgaaagt gttgctaacc gtttacaagg taaaatttta
374    G  V  T  G  L  I  S  G  I  L  E  A  S  K  Q  A  M  F  E  S  V  A  N  R  L  Q  G  K  I  L

1441 gagtgggaaa agcaaaatgg cggtcagaac tattttgata aaggctatga ttctcgttat gctgcttatt tagctaataa cttaaaattt
404    E  W  E  K  Q  N  G  G  Q  N  Y  F  D  K  G  Y  D  S  R  Y  A  A  Y  L  A  N  N  L  K  F

1531 ttgtctgagc taaataaaga gttggaagct gaacgtgtta ttgcaatcac ccaacaacgt tgggataata atattggtga gttagcaggt
434    L  S  E  L  N  K  E  L  E  A  E  R  V  I  A  I  T  Q  Q  R  W  D  N  N  I  G  E  L  A  G

1621 attaccaaat tgggtgaacg cattaagagc ggaaaagctt atgcagatgc ttttgaagat ggcaagaaag ttgaagctgg ttccaatatt
464    I  T  K  L  G  E  R  I  K  S  G  K  A  Y  A  D  A  F  E  D  G  K  K  V  E  A  G  S  N  I

1711 actttggatg ctaaaactgg tatcatagac attagtaatt caaatgggaa aaaaacgcaa gcgttgcatt tcacttcgcc tttgttaaca
494    T  L  D  A  K  T  G  I  I  D  I  S  N  S  N  G  K  K  T  Q  A  L  H  F  T  S  P  L  L  T

1801 gcaggaactg aatcacgtga acgtttaact aatggtaaat actcttatat taataagtta aaattcggac gtgtaaaaaa ctggcaagtt
524    A  G  T  E  S  R  E  R  L  T  N  G  K  Y  S  Y  I  N  K  L  K  F  G  R  V  K  N  W  Q  V

1891 acagatggag aggctagttc taaattagat ttctctaaag ttattcagcg tgtagccgag acagaaggca cagacgagat tggtctaata
554    T  D  G  E  A  S  S  K  L  D  F  S  K  V  I  Q  R  V  A  E  T  E  G  T  D  E  I  G  L  I

1981 gtaaatgcaa aagctggcaa tgacgatatc tttgttggtc aaggtaaaat gaatattgat ggtggagatg gacacgatcg tgtctttat
584    V  N  A  K  A  G  N  D  D  I  F  V  G  Q  G  K  M  N  I  D  G  G  D  G  H  D  R  V  F  Y

2071 agtaaagacg gaggatttgg taatattact gtagatggta cgagtgcaac agaagcaggc agttatacag ttaatcgtaa ggttgctcga
614    S  K  D  G  G  F  G  N  I  T  V  D  G  T  S  A  T  E  A  G  S  Y  T  V  N  R  K  V  A  R

2161 ggtgatatct accatgaagt tgtgaagcgt caagaaacca aggtgggtaa acgtactgaa actatccagt atcgtgatta tgaattaaga
644    G  D  I  Y  H  E  V  V  K  R  Q  E  T  K  V  G  K  R  T  E  T  I  Q  Y  R  D  Y  E  L  R

2251 aaagttgggt atggttatca gtctaccgat aatttgaaat cagtagaaga agtaattggt tctcaattta atgatgtatt caaaggttct
674    K  V  G  Y  G  Y  Q  S  T  D  N  L  K  S  V  E  E  V  I  G  S  Q  F  N  D  V  F  K  G  S

2341 aaattcaacg acatattcca tagtggtgaa ggtgatgatt tactcgatgg tggtgctggt gacgaccgct tgtttggtgg taaaggcaac
704    K  F  N  D  I  F  H  S  G  E  G  D  D  L  L  D  G  G  A  G  D  D  R  L  F  G  G  K  G  N
```

Figure 5

```
2431  gatcgacttt ctggagatga aggcgatgat ttactcgatg gcggttctgg tgatgatgta ttaaatggtg gtgctggtaa tgatgtctat
 734    D  R  L   S  G  D   E  G  D  D   L  L  D   G  G  S   G  D  D  V   L  N  G   G  A  G   N  D  V  Y

2521  atctttcgga aaggtgatgg taatgatact ttgtacgatg gcacgggcaa tgataaatta gcatttgcag atgcaaatat atctgatatt
 764    I  F  R   K  G  D   G  N  D  T   L  Y  D   G  T  G   N  D  K  L   A  F  A   D  A  N   I  S  D  I

2611  atgattgaac gtaccaaaga gggtattata gttaaacgaa atgatcattc aggtagtatt aacataccaa gatggtacat aacatcaaat
 794    M  I  E   R  T  K   E  G  I  I   V  K  R   N  D  H   S  G  S  I   N  I  P   R  W  Y   I  T  S  N

2701  ttacaaaatt atcaaagtaa taaaacagat cataaaattg agcaactaat tggtaaagat ggtagttata tcacttccga tcaaattgat
 824    L  Q  N   Y  Q  S   N  K  T  D   H  K  I   E  Q  L   I  G  K  D   G  S  Y   I  T  S   D  Q  I  D

2791  aaaattttgc aagataagaa agatggtaca gtaattacat ctcaagaatt gaaaaagctt gctgatgaga ataagagcca aaaattatct
 854    K  I  L   Q  D  K   K  D  G  T   V  I  T   S  Q  E   L  K  K  L   A  D  E   N  K  S   Q  K  L  S

2881  gcttcggaca ttgcaagtag cttaaataag ctagttgggt caatggcact atttggtaca gcaaatagtg tgagttctaa cgccttacag
 884    A  S  D   I  A  S   S  L  N  K   L  V  G   S  M  A   L  F  G  T   A  N  S   V  S  S   N  A  L  Q

2971  ccaattacac aaccaactca aggaattttg gctccaagtg tttagtgatt taatttacta gacaatatca ccacccatat cattggttat
 914    P  I  T   Q  P  T   Q  G  I  L   A  P  S   V  -

3061  agattatgaa actagtgata tgggtggtga tacttcttta attagactta atttacaaac ccttaatagt aatttagtta tgatagatta
                           M  G  G   D  T  S  L   I  R  L   N  L  Q   T  L  N  S   N  L  V   M  I  D

3151  tgctcaacaa cctgctctat ctgctctggt tatccttgcc aaatactatg gtatttctgc aagtccagca gacattatgc atcgactggc
      Y  A  Q  Q   P  A  L   S  A  L   V  I  L  A   K  Y  Y   G  I  S   A  S  P  A   D  I  M   H  R  L

3241  aaaaaagttg
      A  K  K  L
```

Figure 5 (continued)

SEQUENCE LISTING

<110> The University of Melbourne
Commonwealth Scientific and Industrial Research Organisation

<120> Vaccine antigens of Moraxella

<160> 9

<170> PatentIn Ver. 2.1

<210> 1
<211> 1114
<212> PRT
<213> Moraxella bovis

<400> 1

```
Met Ser Leu Gln Thr Gln Pro Ala Lys Arg Gly Phe Tyr Val Lys Pro
 1               5                  10                  15

Leu Ser Met Ala Cys Met Leu Val Ile Ser Ala Ser Ser Thr Val Ser
            20                  25                  30

Tyr Ala Asn Ser Ala Pro Met Ile Val Asp Ser Gln Tyr Asn Ser Ser
        35                  40                  45

Lys Tyr Ser Phe Tyr Asp Tyr Tyr Leu Asp Phe Leu Lys Arg Phe Arg
    50                  55                  60

Pro Thr Pro Thr Pro Val Pro Ser Pro Val Arg Pro Ala Pro Glu Leu
65                  70                  75                  80

Val Arg Pro Thr Pro Ala Pro Ile Pro Ala Pro Thr Pro Val Pro Thr
                85                  90                  95

Pro Ala Pro Ile Ser Gly Gly Ile Ser Gly Ser Tyr Ile Ala Pro Val
            100                 105                 110

Ser Pro Ser Glu Val Arg Gln Pro Asp Tyr Thr Arg Arg Val Gln Ala
        115                 120                 125

Asn Leu Lys Arg Asn Gln Pro Ala Pro Ser Ala Gly Thr Arg Thr Gly
    130                 135                 140

Tyr Ser Val Met Asp Thr Ser Asn Asn Ser Asn Leu Thr Ser Lys Phe
145                 150                 155                 160

Tyr Gly Thr Thr Glu Asp Gly Tyr Ala Glu Arg Leu Asp Asn Leu Lys
                165                 170                 175

Asn Thr Ile Asp Thr Arg Gln Ala Lys Val Gly Val Ile Asp Thr Gly
            180                 185                 190

Ile Asn Arg Phe Asn Arg Asp Leu Val Gly Ala Asn Val His Asp Thr
        195                 200                 205
```

```
Gln Ile Glu Cys Val Ser Ala Gly Arg Ser Thr Cys Tyr Thr Pro Glu
    210                 215                 220

Asn Asp Ser Gly Ile Val Glu Ile Pro Thr Thr Ser Ala Ser Gly Ser
225                 230                 235                 240

His Gly Asn Gln Met Ala Ala Val Ile Ala Gly Asn Asn Gly Met Thr
                245                 250                 255

Asn Ala Lys Ile Tyr Gly Ser Asp Ser Ile Asp Arg Arg Ser Asn Gly
            260                 265                 270

Gly Asn His Phe Leu Met Met Arg Lys Leu Asn Gln Asp His Gly Val
        275                 280                 285

Lys Ile Phe Asn Asn Ser Trp Gly Ser Asn Asn Thr Asp Gln Trp Tyr
    290                 295                 300

Tyr Asp Ala Gln Arg Leu Asn Tyr Asn Pro Thr Thr Gly Gln Ile Asn
305                 310                 315                 320

Pro Asn Pro Tyr Arg Thr Ser Ile Thr Asn Ala Glu Val Thr Leu Pro
                325                 330                 335

Val Ile His Asp Leu Ile Met Asn Arg Asp Ser Leu Ile Ile Lys Ala
            340                 345                 350

Thr Gly Asn Glu Gly Leu Asn Asp Ala His Asp Glu Asn Leu Ala Pro
        355                 360                 365

Leu Met Asn Ser Asn Phe Lys Lys Gly Phe Ile Thr Val Ser Ser Pro
    370                 375                 380

Arg Glu Asp Phe Gly Lys Ala Asn His Cys Gly Arg Thr Ala Glu Trp
385                 390                 395                 400

Cys Val Ser Ala Thr Ser Ser Thr Gln Asn Tyr Ala Asn Asp Gly Arg
                405                 410                 415

Leu Ser Ser Tyr Lys Gly Thr Ser Pro Ala Thr Ala Arg Val Ser Gly
            420                 425                 430

Thr Ala Val Leu Val Gln Ser Ala Tyr Pro Trp Met Lys Asn Glu Asn
        435                 440                 445

Ile Ser Gln Thr Ile Leu Gly Thr Ala Lys Asp Phe Ser Glu Ile Thr
    450                 455                 460

Ala Asn Ser Pro Asn Gly Tyr Gln Gly Leu Arg Lys Val Ser Arg Leu
465                 470                 475                 480

Pro Ser Gly Tyr Tyr Gly Ser Tyr Tyr Thr Asp Asn Gln Gly Asn Phe
                485                 490                 495

Tyr Val Pro Gly Asn Val Asn Trp Glu Asn Arg Arg Ile Val Ala Asn
            500                 505                 510
```

```
His Asn Gly Lys Asn Ile Thr Trp Glu Asp Gly Trp Gly Leu Leu Asp
        515                 520                 525

Pro Glu Ala Ala Ala Lys Gly Tyr Gly Gly Phe Tyr Trp Asp Asn Val
    530                 535                 540

Glu Leu Asp Thr Lys Gly Thr Pro Leu Ser Val Phe Tyr Asn Asp Leu
545                 550                 555                 560

Lys Gly Asp Lys Gly Phe Thr Lys Lys Gly Glu Gly Lys Leu Val Phe
                565                 570                 575

Thr Gly Asn Asn Ser Tyr Lys Gly Asp Ser Val Ile Glu Gly Gly Ser
            580                 585                 590

Leu Glu Val Asn Gly Asn Asn Gly Gly Ser Thr Met Val Val Lys Gly
        595                 600                 605

Gly Glu Leu Thr Gly Tyr Gly Asn Val Ala Asn Val Arg Gln Thr Gly
    610                 615                 620

Gly Trp Val Asn Asn Glu Gly Asn Leu Asn Ile Arg Gly Asp Tyr Asn
625                 630                 635                 640

Ile Asn Thr Gln Arg Gly Val Asp Ala Gly Leu Lys Ala Gln Phe Gly
                645                 650                 655

Asn Met Leu Thr Val Asp Gly Lys Ala Lys Leu Gly Gly Thr Leu Asn
            660                 665                 670

Leu Thr Gly Glu Thr Lys Asp Gly Ile Ile Ser Lys Ser Gly Ser Arg
        675                 680                 685

Ser Thr Val Leu Arg Ala Lys Arg Gly Leu Glu Gly Gln Phe Asp Asn
    690                 695                 700

Tyr Arg Ser Ser Asn Pro Leu Phe Glu Val Thr Asn Val Glu Tyr Thr
705                 710                 715                 720

Pro Glu Val Asp Arg Asn Gly Arg Val Val Gly Gly Ser Arg Thr Asn
                725                 730                 735

Asn Asp Val Gln Val Thr Ala Lys Arg Leu Ser Ala Gly Asn Val Val
            740                 745                 750

Tyr Gly Ile Ser Met Asn Asp Ser Gly Ser Arg Val Ala Gln Asn Leu
        755                 760                 765

Asp Lys Val Leu Asn Asp Leu Asp Lys Lys Gln Glu Thr Gln Gly Ser
    770                 775                 780

Leu Thr Ser Asp Glu Lys Gln Phe Ala Asn Arg Val Phe Thr Gly Phe
785                 790                 795                 800

Glu Asn Met Asn Ser Gly Ala Glu Ser Lys Leu Ser Thr Val Ser Thr
                805                 810                 815
```

```
Asn Arg Glu Leu Tyr Lys Leu Asp Pro Thr Phe Tyr Ala Asp Ser Ala
            820                 825                 830

Leu Asn Ala Val Glu Asp Ser Ala Asn His Ala Thr Glu Phe Gly Lys
        835                 840                 845

Arg Val Ser Ala Pro Arg Gly Val Trp Gly Asn Ile Ser His His Asp
    850                 855                 860

Tyr Asp Val Glu Leu Glu His Ala Thr Ser Ala Arg Lys Gly Asn Asn
865                 870                 875                 880

Ile Ser Val Gly Ala Ser Thr Gln Thr Ala Ala Asp Ile Ser Val Gly
                885                 890                 895

Ala Gln Leu Asp Val Ser Lys Leu Asp Leu Glu Glu Ser Val Tyr Gly
            900                 905                 910

Ile Gly Asn Lys Thr Lys Thr Asp Ser Ile Gly Leu Thr Val Gly Ala
        915                 920                 925

Ser Lys Lys Leu Gly Asp Ala Tyr Leu Ser Gly Trp Val Lys Gly Ala
    930                 935                 940

Lys Val Asp Thr Glu Ala Asn Arg Gly Glu Asn Ser Asn Lys Val Glu
945                 950                 955                 960

Tyr Asn Gly Lys Leu Tyr Gly Ala Gly Ile Gln Ala Gly Thr Asn Ile
                965                 970                 975

Asp Thr Ala Ser Gly Val Ser Val Gln Pro Tyr Ala Phe Val Asn His
            980                 985                 990

Gln Gln Tyr Lys Asn Asp Gly Ser Phe Asn Asp Gly Leu Asn Val Val
        995                 1000                1005

Asp Asp Ile Glu Ala Lys Gln Thr Gln Val Gly Val Gly Ala Asp Met
   1010                1015                1020

Val Phe Gln Ala Thr Pro Ala Leu Gln Leu Thr Gly Gly Val Gln Val
1025                1030                1035                1040

Ala His Ala Val Ser Arg Asp Thr Asn Leu Asp Thr Arg Tyr Val Gly
                1045                1050                1055

Thr Ala Thr Asp Val Gln Tyr Gly Thr Trp Asp Thr Asp Lys Thr Lys
            1060                1065                1070

Trp Ser Ala Lys Val Gly Ala Asn Tyr Asn Val Thr Pro Asn Ser Gln
        1075                1080                1085

Val Gly Leu Asn Tyr Ser Tyr Thr Gly Ser Gly Asp Ser Asp Ala Ser
   1090                1095                1100

Gln Val Gly Val Ser Phe Thr Ser Lys Phe
1105                1110
```

<210> 2
<211> 4384
<212> DNA
<213> Moraxella bovis

<400> 2
ttctcatgtt tgacagctta tcatcgataa gctttaatgc ggtagtttat cacagttaaa 60
ttgctaacgc agtcaggcac cgtgtatgaa atctaacaat gcgctcatcg tcatcctcgg 120
caccgtcacc ctggatgctg taggcatagg cttggttatg ccggtactgc cgggcctctt 180
gcgggatatc gtccattccg acagcatcgc cagtcactat ggcgtgctgc tagcgctata 240
tgcgttgatg caatttctat gcgcacccgt tctcggagca ctgtccgacc gctttggccg 300
ccgcccagtc ctgctcgctt cgctacttgg agccactatc gactacgcga tcatggcgac 360
cacacccgtc ctgtggatca ataattaatg aacatatata ctctatttaa tatttcttat 420
ttattcgtaa tattgccata aaaataatac attatttcta tattaactaa actgttaata 480
tttgtaaata ataaacattt gtttatctaa aaaaataaat aatataaatc aagcaattac 540
aatcttattt ttgaaaatac aataatactg caattgctta atctagacat taagtttatt 600
tttgattaaa attgccaaaa cttgtgtaaa taagtttcac cgaattgata ctttaagggt 660
atcaatattg caacatggta aatgattgct atgttgttgg gcattgcata aattgtctat 720
aataacttgt tatggatgat tgatggcaat gataaactta gtgacaatga taaacgcaaa 780
gaggtgtaat atgtcattac aaactcaacc tgccaagaga gggttctatg ttaagccttt 840
aagtatggct tgcatgctgg taattagtgc tagtagtacg gtaagttatg ccaactcagc 900
tccaatgatt gttgattcac agtacaatag ttctaaatac tctttctacg attactattt 960
agatttcctt aaacgtttta gaccaactcc aactccagtg ccaagccctg tgagaccggc 1020
tcctgaactc gttcgtccga ccccagcccc gattccggct ccaacgcctg tgccaacacc 1080
ggcaccaatt agtggcggta tatcaggtag ctatattgct ccagtatcgc catcagaggt 1140
gagacagcct gattacacaa gacgcgttca agccaatcta aaacgcaacc aacctgcacc 1200
aagtgctggc acacgtacag gttatagtgt catggatacg tcaaataatt ctaatttgac 1260
atctaaattt tatggcacaa ccgaagatgg ttatgccgag cgtcttgaca acctaaagaa 1320
caccattgat acacgtcaag ccaaagtagg tgtgattgat acaggcatta accgcttcaa 1380
ccgagacttg gttggtgcaa atgtgcatga tacacagatt gagtgtgttt ctgctggacg 1440
ttccacctgc tatacgccag aaaatgattc aggcattgtt gaaatcccaa caacctctgc 1500
tagtggtagt catggcaacc aaatggcggc tgtcatcgct ggtaacaacg gcatgaccaa 1560
cgccaaaatt tacggcagtg acagtattga tcgacgttca aatggtggca accatttctt 1620
gatgatgcgt aagctgaacc aagaccatgg tgtcaagatt tttaacaact cttggggttc 1680
taacaacact gaccaatggt actacgatgc tcagcgccta aattacaatc ctactacagg 1740
acagattaat ccaaatcctt acagaaccag tattaccaat gctgaagtga ctttgcctgt 1800
cattcatgat cttattatga atcgtgactc gcttatcatt aaagcaacag gtaacgaagg 1860
cttgaacgat gctcatgatg aaaacctagc accgctcatg aacagcaact tcaaaaaagg 1920
tttcattact gtctcctcgc ctagagaaga ttttggtaaa gcgaatcatt gtggtcgaac 1980
tgccgaatgg tgtgtatccg caacatcatc tacccaaaat tacgccaacg atggcagact 2040
gagtagctat aagggtacat cacctgcaac cgctcgtgtg tccggcacgg cagtgctcgt 2100
gcaatctgct tatccttgga tgaaaaatga aaatatctct caaaccattt tgggtactgc 2160
caaggatttc tcagagatta ctgccaattc acctaatggc taccaaggac taagaaaggt 2220
tagtagattg ccatctggtt attacggctc ttattacact gacaatcagg gtaatttcta 2280
tgttcctggc aatgtcaatt gggaaaaccg tcgaattgtc gctaatcata acggcaagaa 2340
cattacatgg gaagatggtt ggggtttgtt agatccagaa gcggccgcta aaggttatgg 2400
tggtttctat tgggataatg tggaattaga cactaaaggc acgcctttat ctgtattcta 2460
caatgaccta aaaggtgata aaggctttac caaaaaaggt gaaggtaaac ttgtctttac 2520
tggtaataat agctataaag gcgactctgt catcgagggt ggttcactag aagtaaatgg 2580
taacaacggt ggttcaacca tggttgttaa aggtggtgaa ctaacaggtt atggtaatgt 2640
agctaatgtt cgtcaaacag gtggttgggt taacaacgaa ggtaacctaa acatcagagg 2700
tgactacaac atcaacactc aacgtggcgt ggatgctggt ctaaaagctc aatttggcaa 2760
catgcttacc gtggacggta aggccaaact aggtggtaca ctaaatctaa ctggtgagac 2820
caaagatggt atcatcagca aatcaggtag ccgtagcact gtacttcgtg ctaagcgtgg 2880
tcttgaaggt caatttgaca attatcgttc aagcaaccca ttatttgaag taacaaatgt 2940

tgaatatacg ccagaagtag acagaaatgg cagagtggta ggtggttcac gcacgaacaa 3000
tgacgtgcaa gtaactgcca aacgtctaag tgcaggcaat gttgtttatg gcatcagcat 3060
gaatgacagt ggtagccgtg ttgcacaaaa cctagacaaa gtacttaatg atttagataa 3120
aaaacaagaa acacaaggtt cactgaccag tgatgagaag caatttgcta accgtgtatt 3180
cactggtttt gaaaacatga attctggtgc agaatctaaa ctttctacag taagcaccaa 3240
ccgtgagcta tacaagcttg acccaacttt ctatgctgac agtgcattaa acgcagtaga 3300
agacagtgct aaccatgcaa ccgaatttgg taagcgtgtt agcgccccaa gaggtgtttg 3360
gggtaatatc agtcaccatg attatgatgt agaactagag catgctacaa gtgcacgtaa 3420
aggcaacaac attagtgttg gtgcaagcac tcaaactgca gccgacatta gtgttggtgc 3480
acaacttgat gtaagtaaac ttgacttgga agaatctgtt tatggtattg gcaacaaaac 3540
caaaactgac agcattggct tgactgttgg tgcttctaag aagttgggtg atgcctatct 3600
atcaggttgg gtaaaaggtg ccaaagttga tacagaagcg aaccgtggtg aaaactctaa 3660
caaagttgag tacaatggta agctatatgg tgctggtatc caagcgggta caaacattga 3720
tactgcatcg ggcgtgagtg tacaacctta tgcctttgtt aaccatcagc agtacaaaaa 3780
cgatggtagc ttcaatgacg gtcttaacgt tgttgacgac atcgaagcaa aacaaactca 3840
ggtaggtgtg ggtgctgata tggtgttcca agcaacacct gctctacagc ttactggtgg 3900
tgtgcaagtt gctcacgctg ttagccgtga caccaaccta gacactcgct atgttggtac 3960
agcgacagat gtacagtatg gcacttggga tactgacaaa accaaatggt cagccaaggt 4020
tggtgctaac tataatgtga caccaaacag ccaagtgggt ctaaactaca gctacacagg 4080
tagtggcgat tcagatgctt cccaagtggg tgtgagcttc accagcaagt tctaattcat 4140
taataaggca acaaaaaaca gcacaatttc ggttgtgctg ttttttgtga tgccgagcgt 4200
aaaattttcc caaaaaaagc gtgataatta ccacgctttt ttattgcata ttgcaaaata 4260
gtattgcatt tatgggttgt taagcaaccc gtccaaatac cccctaaaca actccacccc 4320
aatcggtgct aacttgtttt gccacaggct cgtcaatgtg tcggcatcat caaccattac 4380
cgac 4384

<210> 3
<211> 616
<212> PRT
<213> Moraxella bovis

<400> 3
Met Lys Lys Ser Ala Phe Ala Lys Tyr Ser Ala Leu Ala Leu Met Val
1 5 10 15

Gly Met Cys Leu His Thr Ala Tyr Ala Lys Glu Phe Ser Gln Val Ile
20 25 30

Ile Phe Gly Asp Ser Leu Ser Asp Thr Gly Arg Leu Lys Asp Met Val
35 40 45

Ala Arg Lys Asp Gly Thr Leu Gly Asn Thr Leu Gln Pro Ser Phe Thr
50 55 60

Thr Asn Pro Asp Pro Val Trp Ser Ser Leu Phe Ala Gln Ser Tyr Gly
65 70 75 80

Lys Thr Ala Ser Ala Asn Thr Pro Tyr Asn Pro Thr Gly Thr Asn Tyr
85 90 95

Ala Val Gly Gly Ala Arg Ser Gly Ser Glu Val Asn Trp Asn Gly Phe
100 105 110

Val Asn Val Pro Ser Thr Lys Thr Gln Ile Thr Asp His Leu Thr Ala
115 120 125

```
Thr Gly Gly Lys Ala Asp Pro Asn Thr Leu Tyr Ala Ile Trp Ile Gly
    130                 135                 140

Ser Asn Asp Leu Ile Ser Ala Ser Gln Ala Thr Thr Thr Ala Glu Ala
145                 150                 155                 160

Gln Asn Ala Ile Lys Gly Ala Val Thr Arg Thr Val Ile Asp Ile Glu
                165                 170                 175

Thr Leu Asn Glu Ala Gly Ala Thr Thr Ile Leu Val Pro Asn Val Pro
            180                 185                 190

Asp Leu Ser Leu Thr Pro Arg Ala Ile Tyr Gly Glu Ser Leu Met Ala
        195                 200                 205

Gly Val Gln Asp Lys Ala Lys Leu Ala Ser Ser Leu Tyr Asn Ser Gly
    210                 215                 220

Leu Phe Glu Ala Leu Asn Gln Ser Thr Ala Asn Ile Ile Pro Ala Asn
225                 230                 235                 240

Thr Phe Ala Leu Leu Gln Glu Ala Thr Thr Asn Lys Glu Ala Phe Gly
                245                 250                 255

Phe Lys Asn Thr Gln Gly Val Ala Cys Gln Met Pro Ala Arg Thr Thr
            260                 265                 270

Gly Ala Asp Asp Val Ala Ser Thr Ser Leu Ala Cys Thr Lys Ala Asn
        275                 280                 285

Leu Ile Glu Asn Gly Ala Asn Asp Thr Tyr Ala Phe Ala Asp Asp Ile
    290                 295                 300

His Pro Ser Gly Arg Thr His Arg Ile Leu Ala Gln Tyr Tyr Arg Ser
305                 310                 315                 320

Ile Met Asp Ala Pro Thr His Met Gly Lys Leu Ser Gly Glu Leu Val
                325                 330                 335

Lys Thr Gly Ser Ala His Asp Arg His Val Tyr Arg Gln Leu Asp Arg
            340                 345                 350

Leu Ser Gly Ser Gln His Ser Ile Trp Ala Asn Val His Ala Ser Asp
        355                 360                 365

Arg Thr Asp Pro Thr Thr Gln Ile Gly Leu Asp Val Ala Gly Ser Ser
    370                 375                 380

Ser His Thr Gly Ala Tyr Leu Ser His Gln Asn Gln Asp Tyr Val Leu
385                 390                 395                 400

Asp Asp Thr Leu Ser Ser Asp Val Lys Thr Ile Gly Met Gly Leu Tyr
                405                 410                 415

His Arg His Asp Ile Gly Asn Val Arg Leu Lys Gly Val Ala Gly Ile
            420                 425                 430
```

```
Asp Arg Leu Ser Val Asp Thr His Arg His Ile Asp Trp Glu Gly Ala
        435                 440                 445

Ser Arg Ser His Thr Ala Asp Thr Thr Ala Arg Arg Phe His Ala Gly
    450                 455                 460

Leu Gln Ala Ser Tyr Gly Ile Asp Met Gly Lys Ala Thr Val Arg Pro
465                 470                 475                 480

Leu Ile Gly Val His Ala Gln Lys Val Lys Val Arg Asp Leu Val Glu
                485                 490                 495

Asn Glu Pro Thr Leu Ser Thr Ala Met Arg Phe Gly Glu Gln Glu Gln
            500                 505                 510

Lys Ser Leu Gln Gly Glu Ile Gly Val Asp Val Ala Tyr Pro Ile Ser
        515                 520                 525

Pro Ala Leu Thr Leu Thr Gly Gly Ile Ala His Ala His Glu Phe Asn
    530                 535                 540

Asp Asp Glu Arg Thr Ile Asn Ala Thr Leu Thr Ser Ile Arg Glu Tyr
545                 550                 555                 560

Thr Lys Gly Phe Asn Thr Ser Val Ser Thr Asp Lys Ser His Ala Thr
                565                 570                 575

Thr Ala His Leu Gly Val Gln Gly Gln Leu Gly Lys Ala Asn Ile His
            580                 585                 590

Ala Gly Val His Ala Thr His Gln Asp Ser Asp Thr Asp Val Gly Gly
        595                 600                 605

Ser Leu Gly Val Arg Leu Met Phe
    610                 615
```

<210> 4
<211> 2110
<212> DNA
<213> Moraxella bovis

<400> 4

```
tgacaaataa ttgggcattg ggcagataac ccatcaaaga cccaaagcaa cccataaatc 60
aaaaaaacac ttgtaatttt tgtaatatct tgttacactt tacaagtgtt tttactttga 120
aagcaactca gagagtaata atgaaaaaat ccgcctttgc caaatactca gcacttgccc 180
taatgyttgg gatgtgcctg cacaccgctt acgccaagga gtttagccaa gtcatcattt 240
ttggggacag cttgtccgat acaggtcgcc taaaagatat ggtcgcccga aaagatggca 300
cccttggcaa caccttacag ccatctttta ccaccaaccc cgaccctgta tggtcaagct 360
tatttgccca aagttatggc aaaaccgcca gtgccaacac gccctacaat cccactggca 420
ctaactatgc cgtgggcgga gctcgctctg gctcggaggt caattggaat ggttttgtga 480
atgtaccctc caccaaaacg caaatcaccg accatttgac cgccacaggt ggcaaagccg 540
accctaatac cctgtatgcc atttggattg gctctaatga cttaatttca gcttctcaag 600
ccaccacaac agccgaagcc caaaacgcca ttaaaggtgc ggtaactcgc accgtgatag 660
acatcgaaac actcaatcaa gcaggggcga caaccatttt ggtgccaaat gtgcctgatt 720
tgagcctcac gccccgagcc atctatggcg aaagcctcat ggcaggcgtg caagacaaag 780
```

ccaaactcgc ctcaagtctg tataatagcg gtctgtttga agcattaaat caatccaccg 840
ccaacatcat ccctgccaac acctttgccc tactccaaga agcgaccaca aataaagaag 900
cctttggttt taaaaacacg caaggcgtgg cgtgtcaaat gcccgctcgt accacagggg 960
cggatgatgt ggcttctact tccttggcat gtaccaaagc caatcttata gaaaacgggg 1020
caaatgacac ctacgccttt gccgatgaca ttcacccatc gggacgcacg caccgcattt 1080
tggcacagta ttaccgttct atcatggacg cccctactca catgggtaaa ctctcaggcg 1140
agcttgtcaa aacaggttca gcccacgacc gtcatgttta ccgtcagctt gacaggctta 1200
gtggctcaca gcacagcatt tgggcaaacg tccatgccag cgaccgtacc gaccccacca 1260
cccaaatcgg cttggacgtg gcaggttcat caagccatac aggggcgtat ctgagccacc 1320
aaaaccaaga ttatgtgctg gatgacaccc tatcatcaga tgtcaaaacc attggcatgg 1380
ggctgtatca tcgccatgac atcggcaatg tccgtctaaa aggcgtggca ggtatcgacc 1440
gacttagcgt ggatacgcac cgccatatcg actgggaggg ggcaagccgt tcgcacacgg 1500
cagacaccac cgccagacgt tttcatgcag ggctacaagc cagctatggc atagacatgg 1560
gcaaagccac cgtgcgtccg cttatcggcg tacatgccca aaaagtcaaa gtgcgtgatt 1620
tggtagagaa tgagcctacc ctatccaccg ccatgcgttt tggcgagcaa gaacaaaagt 1680
ccctacaagg cgagattggc gtcgatgtgg cttatccgat tagccctgct ttgactctga 1740
cgggcggtat cgctcacgct catgagttta acgatgatga acgcaccatt aatgccactt 1800
taacctccat tcgtgaatac acgaagggct ttaatacaag cgttagcacc gacaaatctc 1860
acgccaccac cgctcatctg ggcgtacaag ggcaacttgg caaggcaaat attcatgcag 1920
gcgttcacgc cacccaccaa gacagcgata cagacgtggg tggttcgctt ggggttcgct 1980
tgatgttttg attggctttt aaagataaaa agtggtatca tgccactttt tattttgcca 2040
aaaatctatg tttgagtaca tcaaagcctt tcacatcatc gccatgcgat gataagctgt 2100
caaacatgag 2110

<210> 5
<211> 927
<212> PRT
<213> Moraxella bovis

<400> 5
Met Ser Asn Ile Asn Val Ile Lys Ser Asn Ile Gln Ala Gly Leu Asn
1 5 10 15

Ser Thr Lys Ser Gly Leu Lys Asn Leu Tyr Leu Ala Ile Pro Lys Asp
20 25 30

Tyr Asp Pro Gln Lys Gly Gly Thr Leu Asn Asp Phe Ile Lys Ala Ala
35 40 45

Asp Glu Leu Gly Ile Ala Arg Leu Ala Glu Glu Pro Asn His Thr Glu
50 55 60

Thr Ala Lys Lys Ser Val Asp Thr Val Asn Gln Phe Leu Ser Leu Thr
65 70 75 80

Gln Thr Gly Ile Ala Ile Ser Ala Thr Lys Leu Glu Lys Phe Leu Gln
85 90 95

Lys His Ser Thr Asn Lys Leu Ala Lys Gly Leu Asp Ser Val Glu Asn
100 105 110

Ile Asp Arg Lys Leu Gly Lys Ala Ser Asn Val Leu Ser Thr Leu Ser
115 120 125

Ser Phe Leu Gly Thr Ala Leu Ala Gly Ile Glu Leu Asp Ser Leu Ile
130 135 140

Lys Lys Gly Asp Ala Ala Pro Asp Ala Leu Ala Lys Ala Ser Ile Asp
145 150 155 160

Leu Ile Asn Glu Ile Ile Gly Asn Leu Ser Gln Ser Thr Gln Thr Ile
165 170 175

Glu Ala Phe Ser Ser Gln Leu Ala Lys Leu Gly Ser Thr Ile Ser Gln
180 185 190

Ala Lys Gly Phe Ser Asn Ile Gly Asn Lys Leu Gln Asn Leu Asn Phe
195 200 205

Ser Lys Thr Asn Leu Gly Leu Glu Ile Ile Thr Gly Leu Leu Ser Gly
210 215 220

Ile Ser Ala Gly Phe Ala Leu Ala Asp Lys Asn Ala Ser Thr Gly Lys
225 230 235 240

Lys Val Ala Ala Gly Phe Glu Leu Ser Asn Gln Val Ile Gly Asn Val
245 250 255

Thr Lys Ala Ile Ser Ser Tyr Val Leu Ala Gln Arg Val Ala Ala Gly
260 265 270

Leu Ser Thr Thr Gly Ala Val Ala Ala Leu Ile Thr Ser Ser Ile Met
275 280 285

Leu Ala Ile Ser Pro Leu Ala Phe Met Asn Ala Ala Asp Lys Phe Asn
290 295 300

His Ala Asn Ala Leu Asp Glu Phe Ala Lys Gln Phe Arg Lys Phe Gly
305 310 315 320

Tyr Asp Gly Asp His Leu Leu Ala Glu Tyr Gln Arg Gly Val Gly Thr
325 330 335

Ile Glu Ala Ser Leu Thr Thr Ile Ser Thr Ala Leu Gly Ala Val Ser
340 345 350

Ala Gly Val Ser Ala Ala Ala Val Gly Ser Ala Val Gly Thr Pro Ile
355 360 365

Ala Leu Leu Val Ala Gly Val Thr Gly Leu Ile Ser Gly Ile Leu Glu
370 375 380

Ala Ser Lys Gln Ala Met Phe Glu Ser Val Ala Asn Arg Leu Gln Gly
385 390 395 400

Lys Ile Leu Glu Trp Glu Lys Gln Asn Gly Gly Gln Asn Tyr Phe Asp
405 410 415

Lys Gly Tyr Asp Ser Arg Tyr Ala Ala Tyr Leu Ala Asn Asn Leu Lys
420 425 430

Phe Leu Ser Glu Leu Asn Lys Glu Leu Glu Ala Glu Arg Val Ile Ala
435 440 445

```
Ile Thr Gln Gln Arg Trp Asp Asn Asn Ile Gly Glu Leu Ala Gly Ile
    450                 455                 460

Thr Lys Leu Gly Glu Arg Ile Lys Ser Gly Lys Ala Tyr Ala Asp Ala
465                 470                 475                 480

Phe Glu Asp Gly Lys Lys Val Glu Ala Gly Ser Asn Ile Thr Leu Asp
                485                 490                 495

Ala Lys Thr Gly Ile Ile Asp Ile Ser Asn Ser Asn Gly Lys Lys Thr
            500                 505                 510

Gln Ala Leu His Phe Thr Ser Pro Leu Leu Thr Ala Gly Thr Glu Ser
        515                 520                 525

Arg Glu Arg Leu Thr Asn Gly Lys Tyr Ser Tyr Ile Asn Lys Leu Lys
    530                 535                 540

Phe Gly Arg Val Lys Asn Trp Gln Val Thr Asp Gly Glu Ala Ser Ser
545                 550                 555                 560

Lys Leu Asp Phe Ser Lys Val Ile Gln Arg Val Ala Glu Thr Glu Gly
                565                 570                 575

Thr Asp Glu Ile Gly Leu Ile Val Asn Ala Lys Ala Gly Asn Asp Asp
            580                 585                 590

Ile Phe Val Gly Gln Gly Lys Met Asn Ile Asp Gly Gly Asp Gly His
        595                 600                 605

Asp Arg Val Phe Tyr Ser Lys Asp Gly Gly Phe Gly Asn Ile Thr Val
    610                 615                 620

Asp Gly Thr Ser Ala Thr Glu Ala Gly Ser Tyr Thr Val Asn Arg Lys
625                 630                 635                 640

Val Ala Arg Gly Asp Ile Tyr His Glu Val Val Lys Arg Gln Glu Thr
                645                 650                 655

Lys Val Gly Lys Arg Thr Glu Thr Ile Gln Tyr Arg Asp Tyr Glu Leu
            660                 665                 670

Arg Lys Val Gly Tyr Gly Tyr Gln Ser Thr Asp Asn Leu Lys Ser Val
        675                 680                 685

Glu Glu Val Ile Gly Ser Gln Phe Asn Asp Val Phe Lys Gly Ser Lys
    690                 695                 700

Phe Asn Asp Ile Phe His Ser Gly Glu Gly Asp Asp Leu Leu Asp Gly
705                 710                 715                 720

Gly Ala Gly Asp Asp Arg Leu Phe Gly Gly Lys Gly Asn Asp Arg Leu
                725                 730                 735

Ser Gly Asp Glu Gly Asp Asp Leu Leu Asp Gly Gly Ser Gly Asp Asp
            740                 745                 750
```

Val Leu Asn Gly Gly Ala Gly Asn Asp Val Tyr Ile Phe Arg Lys Gly
755 760 765

Asp Gly Asn Asp Thr Leu Tyr Asp Gly Thr Gly Asn Asp Lys Leu Ala
770 775 780

Phe Ala Asp Ala Asn Ile Ser Asp Ile Met Ile Glu Arg Thr Lys Glu
785 790 795 800

Gly Ile Ile Val Lys Arg Asn Asp His Ser Gly Ser Ile Asn Ile Pro
805 810 815

Arg Trp Tyr Ile Thr Ser Asn Leu Gln Asn Tyr Gln Ser Asn Lys Thr
820 825 830

Asp His Lys Ile Glu Gln Leu Ile Gly Lys Asp Gly Ser Tyr Ile Thr
835 840 845

Ser Asp Gln Ile Asp Lys Ile Leu Gln Asp Lys Lys Asp Gly Thr Val
850 855 860

Ile Thr Ser Gln Glu Leu Lys Lys Leu Ala Asp Glu Asn Lys Ser Gln
865 870 875 880

Lys Leu Ser Ala Ser Asp Ile Ala Ser Ser Leu Asn Lys Leu Val Gly
885 890 895

Ser Met Ala Leu Phe Gly Thr Ala Asn Ser Val Ser Ser Asn Ala Leu
900 905 910

Gln Pro Ile Thr Gln Pro Thr Gln Gly Ile Leu Ala Pro Ser Val
915 920 925

<210> 6
<211> 3231
<212> DNA
<213> Moraxella bovis

<400> 6
atgagaacgt tattttcaga tgaattgttt agagcgattc gtgtagatgg aaattcatcg 60
catggtaaga tatctgaatt ttatggaaag tctgttgatt caaaattagc ctcaagaata 120
tttgcacaat atcacgaaga tttgacgagc aaattgtcaa ctcagaataa ttttattata 180
tctaaagata attaatacaa ccttttctaa cacaacgagg agagacatat tatgtccaat 240
ataaatgtaa ttaaatctaa tattcaagca ggcttgaatt caacaaagtc tggattaaaa 300
aatctttact tggctattcc caaagattat gatccgcaaa aaggtgggac tttaaatgat 360
tttattaaag ctgctgatga attaggtatt gctcgtttag cagaagagcc taatcacact 420
gaaacagcaa aaaaatctgt tgacacagta aatcagtttc tctctctcac acaaactggt 480
attgctattt ctgcaacaaa attagaaaag ttcttacaaa aacattctac caataagtta 540
gccaaagggt tagacagtgt agaaaatatt gatcgtaaat taggtaaagc aagtaatgta 600
ttatcaacat taagctcttt tttgggcact gcattagcgg gtatagaact tgattcttta 660
atcaaaaaag gtgatgctgc acctgatgct ttggctaaag ctagtattga cttgattaat 720
gagataattg gtaatctatc tcagagtact caaacgattg aagcattttc ttcacagtta 780
gcaaagttag gttctactat atcgcaggct aaaggcttct ctaatatagg aaacaagttg 840
caaaacttaa atttttctaa aacaaatctt ggtttggaaa taattactgg tttgctatca 900

ggcatttctg caggctttgc tttagcggat aaaaatgcat cgactggcaa aaaagttgct 960
gcaggttttg aattaagcaa tcaagttatt ggtaatgtaa caaaagcaat ttcttcatat 1020
gttttagcac aacgtgttgc tgctggtcta tcaactactg gtgctgttgc tgctttaatt 1080
acttcatcga ttatgttggc aattagtcct ttggcattta tgaatgcagc agataaattc 1140
aatcatgcta atgctcttga tgagtttgca aaacaattcc gaaaatttgg ctatgatggg 1200
gatcatttat tggctgaata tcagcgtggt gtgggtacta ttgaagcttc attaactaca 1260
attagtacgg cattaggtgc agtttctgct ggtgtttccg ctgctgctgt aggatctgct 1320
gttggtacac cgattgcact attagttgca ggtgttacag gattgatctc tggaatttta 1380
gaagcgtcta aacaggcaat gtttgaaagt gttgctaacc gtttacaagg taaaatttta 1440
gagtgggaaa agcaaaatgg cggtcagaac tattttgata aaggctatga ttctcgttat 1500
gctgcttatt tagctaataa cttaaaattt ttgtctgagc taaataaaga gttggaagct 1560
gaacgtgtta ttgcaatcac ccaacaacgt tgggataata atattggtga gttagcaggt 1620
attaccaaat tgggtgaacg cattaagagc ggaaaagctt atgcagatgc ttttgaagat 1680
ggcaagaaag ttgaagctgg ttccaatatt actttggatg ctaaaactgg tatcatagac 1740
attagtaatt caaatgggaa aaaaacgcaa gcgttgcatt tcacttcgcc tttgttaaca 1800
gcaggaactg aatcacgtga acgtttaact aatggtaaat actcttatat taataagtta 1860
aaattcggac gtgtaaaaaa ctggcaagtt acagatggag aggctagttc taaattagat 1920
ttctctaaag ttattcagcg tgtagccgag acagaaggca cagacgagat tggtctaata 1980
gtaaatgcaa aagctggcaa tgacgatatc tttgttggtc aaggtaaaat gaatattgat 2040
ggtggagatg gacacgatcg tgtcttctat agtaaagacg gaggatttgg taatattact 2100
gtagatggta cgagtgcaac agaagcaggc agttatacag ttaatcgtaa ggttgctcga 2160
ggtgatatct accatgaagt tgtgaagcgt caagaaacca aggtgggtaa acgtactgaa 2220
actatccagt atcgtgatta tgaattaaga aaagttgggt atggttatca gtctaccgat 2280
aatttgaaat cagtagaaga agtaattggt tctcaattta atgatgtatt caaaggttct 2340
aaattcaacg acatattcca tagtggtgaa ggtgatgatt tactcgatgg tggtgctggt 2400
gacgaccgct tgtttggtgg taaaggcaac gatcgacttt ctggagatga aggcgatgat 2460
ttactcgatg gcggttctgg tgatgatgta ttaaatggtg gtgctggtaa tgatgtctat 2520
atctttcgga aaggtgatgg taatgatact ttgtacgatg gcacgggcaa tgataaatta 2580
gcatttgcag atgcaaatat atctgatatt atgattgaac gtaccaaaga gggtattata 2640
gttaaacgaa atgatcattc aggtagtatt aacataccaa gatggtacat aacatcaaat 2700
ttacaaaatt atcaaagtaa taaaacagat cataaaattg agcaactaat tggtaaagat 2760
ggtagttata tcacttccga tcaaattgat aaaattttgc aagataagaa agatggtaca 2820
gtaattacat ctcaagaatt gaaaaagctt gctgatgaga ataagagcca aaaattatct 2880
gcttcggaca ttgcaagtag cttaaataag ctagttgggt caatggcact atttggtaca 2940
gcaaatagtg tgagttctaa cgccttacag ccaattacac aaccaactca aggaattttg 3000
gctccaagtg tttagtgatt taatttacta gacaatatca ccacccatat cattggttat 3060
agattatgaa actagtgata tgggtggtga tacttcttta attagactta atttacaaac 3120
ccttaatagt aatttagtta tgatagatta tgctcaacaa cctgctctat ctgctctggt 3180
tatccttgcc aaatactatg gtatttctgc aagtccagca gacattatgc a 3231

<210> 7
<211> 17
<212> PRT
<213> Moraxella bovis

<220>
<221> misc_feature
<222> (14) (16)
<223> Xaa = unknown

<400> 7
Lys Glu Phe Ser Gln Val Ile Ile Phe Gly Asp Ser Leu Xaa Asp Xaa
1 5 10 15

Gly

<210> 8
<211> 64
<212> PRT
<213> Moraxella bovis

<400> 8
Met Arg Thr Leu Phe Ser Asp Glu Leu Phe Arg Ala Ile Arg Val Asp
1 5 10 15

Gly Asn Ser Ser His Gly Lys Ile Ser Glu Phe Tyr Gly Lys Ser Val
20 25 30

Asp Ser Lys Leu Ala Ser Arg Ile Phe Ala Gln Tyr His Glu Asp Leu
35 40 45

Thr Ser Lys Leu Ser Thr Gln Asn Asn Phe Ile Ile Ser Lys Asp Asn
50 55 60

<210> 9
<211> 57
<212> PRT
<213> Moraxella bovis

<400> 9
Met Gly Gly Asp Thr Ser Leu Ile Arg Leu Asn Leu Gln Thr Leu Asn
1 5 10 15

Ser Asn Leu Val Met Ile Asp Tyr Ala Gln Gln Pro Ala Leu Ser Ala
20 25 30

Leu Val Ile Leu Ala Lys Tyr Tyr Gly Ile Ser Ala Ser Pro Ala Asp
35 40 45

Ile Met His Arg Leu Ala Lys Lys Leu
50 55

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/01048

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C07K 14/22, 16/12; A61K 38/48, 39/095; C12N 15/31

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
ANGIS: SEQ ID Nos. 1, 3, 5
ESPACE: keywords: Moraxella, antigen

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 90/07525, A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA (12 July 1990) see whole document	1-38
A	EP 0 146 523, A2 (NORDEN LABORATORIES) (26 June 1985) see whole document	1-38
A	Journal of Clinical Microbiology, vol. 13, No. 2, (February 1981), pages 269-271, Sandra K. Frank *et al.*, "Hydrolytic Enzymes of *Moraxella bovis*". see whole document	1-38

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 October 2000	23 OCT 2000
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer DAVID GRIFFITHS Telephone No : (02) 6283 2628

INTERNATIONAL SEARCH REPORT

International application No
PCT/AU00/01048

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FEMS Microbiology Letters. Vol. 124 (1994) pages 69-73, F. Mark Billson *et al.*, "A haemolytic cell-free preparation of *Moraxella bovis* confers protection against Infectious Bovine Keratoconjunctivitis". see whole document	1-38
A	American Journal of Veterinary Research, Vol. 51, No. 2 February 1990' pages 191-196, Patricia S. Hoien-Dalen *et al.*, "Comparative characterization of the leukocidic and hemolytic activity of *Moraxella bovis*". see whole document	1-38

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU00/01048

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
EP	146523	DE	3482442	DK	5950/84	ES	538474
		ES	8608320	GR	81194	IE	57878
		JP	60-146831	NZ	210490	PT	79640
		US	4675176	ZA	8409616		
WO	90/07525	AU	50886/90				

END OF ANNEX

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.